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THE JOHNS HOPKINS PRESS
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THE CENTERS OF THE VEGETATIVE NERVOUS SYSTEM[1]

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The exact nature of the influence exerted by the central nervous system upon the viscera is unknown. Nevertheless, the loci from which nervous impulses within the central nervous system modify the activity of the peripheral organs are becoming more circumscribed and the type of activity contributed by such loci is, within the last decade, better understood. The lack of clear cut knowledge concerning these relationships is accounted for by the existence of ganglia between the center and the organ innervated, or in the peripheral organ itself, and the fact that the central pathways are not composed of long single neurones but rather of many short ones. This analysis is further complicated by the ease with which some organs (ureter, arteries) can be excited directly by mechanical stimuli (such as distension), or by chemical influences reaching them through the blood stream. The smooth muscles, the peripheral ganglia, or the distal neurones of the central motor innervation may compensate, within a short time, for the loss suffered by injury to higher centers. Such an analysis is further embarrassed by the existence of both crossed and uncrossed central adjustors, so that a one-sided lesion within a center (for example, one-sided injuries of cortical bladder centers) will not produce a picture of loss of control of internal organs so related to the central nervous system.

The analysis of the results following stimulation of "vegetative centers" presents also certain difficulties. If a definite change in an internal organ, or in a particular form of metabolism, is observed following stimulation of a given locus within the central nervous system, such a change is not sufficient evidence to establish the existence of a "center" in this region, for this effect may be due to the stimulation

[1] Read by invitation at the Phipps Psychiatric Clinic of Johns Hopkins University, Baltimore, Maryland.

of fibers connected anteriorly with more cephalward nuclei. It would be necessary to repeat such a stimulation after degeneration of all fibers starting from higher parts of the central nervous system. Again, such an observed change may not be direct but indirect, that is, the result might be due to the release, by the stimulus, of hormones, which in their turn might be the immediate cause of contraction of smooth muscles far distant from them (for example, the adrenals). Further, if one studies the central regulation of metabolic processes, such as those of water, carbohydrate, fat, or protein, one has to remember the influence that they exert upon each other. If the function of a single organ (*e.g.*, the liver) is changed by injury or stimulation of the central nervous system, those of other organs and, as a consequence, many forms of metabolism may be modified without further intervention of the central nervous system. Therefore, a change of the function of an internal organ, or of a special form of metabolism following an injury or irritation at a given point of the central nervous system, is not yet a sufficient proof that a center of this organ, or of this function, exists in this area. With these difficulties in mind, the writer proposes to outline briefly the present status of our knowledge of the loci of the "centers" of the autonomic nervous system with special reference to his own researches and those of his co-workers.

This consideration of the relation of the central nervous system to the viscera defines the autonomic nervous system, not only as purely motor, as Langley (1922) did, but includes also the *centripetal pathways* from internal organs in the autonomous nervous system. The position of the cell body and the route of entrance into the cord of the visceral afferents is by no means established. Foerster (1926), for instance, believes that the cell body of such afferents may be found not only in dorsal root ganglia, but also in the chain ganglia of the sympathetic chain. Also, he maintains that such afferents may enter the cord directly via the dorsal or the ventral roots (*c.f.* also, Lehmann, 1921), or that they may synapse either within the dorsal root ganglia, or within those of the chain.

After bilateral section of the dorsal roots from the eighth cervical to the fourth thoracic segments, Spiegel and Singer (1927) were not able to observe pain reactions on stimulation of the heart, the aorta, or the stellate ganglion. Thus, they came to the same conclusion for

the thoracic viscera as had Meyer and Froehlich (1922) for the region supplied by the splanchnic nerve: the centripetal impulses from the viscera are conducted by the posterior roots. The cell bodies of those fibers are found within the dorsal root ganglia, as Langley (1903) has pointed out.

In what manner do the axis-cylinders of these cells reach the central nervous system? Daniélopolu (1924), following the histological findings of Dogiel (1895), held that the axones of such cells synapse with the somatic sensory cells within the spinal ganglia, whereas Müller (1931) suggested that these axones probably enter the spinal cord directly. Daniélopolu thought that it might be possible to interrupt the centripetal impulses from the heart and aorta by cutting the nervi intercostales of the respective segments, producing a degeneration in the cells of the dorsal root ganglia. Hashimoto and Spiegel (1930) resected the first four intercostal nerves in dogs. In others, they also cut the plexus brachialis on the same side. A few weeks later, they stimulated the nerve fibers of the stellate ganglion of each side and watched for evidences of pain reaction. These pain reactions were very marked on both sides, perhaps only a little weaker on the operated side. This result suggests certainly that the majority of the fibers carrying visceral sensory impulses leaving the spinal ganglia enter the spinal cord directly.

There are two groups of systems in the *spinal cord* that conduct painful stimuli from the viscera, one composed of long neurones and another of short ones. The first group joins the old tractus spino-thalamicus [Chordotomy in man (Spiller, Schüller, 1910), and animal experiments (Spiegel and Bernis 1925)]. The last two authors have demonstrated that conduction follows both the crossed and the uncrossed spino-thalamic tract. Pain impulses from the arteries of the leg (Spiegel, 1923) and from the regions either of the pelvic nerve (Abuel working with Spiegel, 1930), or of the stellate ganglion (Hashimoto, 1930) were carried by these two divisions. Studying the pain conduction from somatic afferent nerves, Schiff (1894), Rothmann (1906), Karplus and Kreidl (1925) made crossed hemisections in different parts of the spinal cord and showed that even after such an operation stimuli from afferent somatic nerves were conducted to the higher centers. These experiments suggest that a second pathway

exists for the conduction of pain from the surface of the body, a pathway consisting of short neurones which synapse frequently in the central grey substance. Similar experiments were made by Bernis and Spiegel (1925) using the splanchnic nerve as the afferent pathway. Because the hemisections had to be made above the sixth thoracic segment, nothing definite was observed. A result more comparable with that of the somatic pain afferents could be expected while using the pelvic nerve as the afferent route for stimuli entering the spinal cord. Abuel (working with Spiegel, 1930) stimulated this nerve and demonstrated, indeed, pain conduction after double hemisection, first in the lumbar cord on one side, and secondly, in the cervical cord in the other. Conduction by chains of short neurones is, therefore, possible for vegetative centripetal impulses (here, of course, pain), if the number of segments of the cord between the two hemisections is great enough. The importance of short neurones for the conduction of visceral afferent impulses within the spinal cord has also been shown by Davis, Hart, and Crain (1929).

Knowledge of the relationships of such pain conduction to higher centers is practically nil. Bernis and Spiegel (1925) showed that stimulation of the afferent end of the splanchnic changes the respiration in decerebrate preparations. Evidently there is some connection between these afferent pathways and the "center" of respiration, but nothing is known of their intimate relations, either with the thalamus or with the cerebral cortex. One might expect a more intimate knowledge of the centripetal pathways from the viscera, if the study of visceral afferents be not confined to those that result in painful reactions. The investigation of reflexes resulting from the electrical stimuli of afferent nerve fibers from the viscera (see Carlson and Luckhardt, 1920, 1921), and of sensation from the serous (Hoffmann 1920; Capps, 1911, 1922) and mucous membranes (Carlson, 1916; Hurst, 1911), and finally of sensation from the stretching of hollow organs (Hurst, 1911; Head, 1922; Payne and Poulton, 1927; Learmouth, 1931) might be a valuable help in further studies of the afferent visceral impulses within the central nervous system.

The *centrifugal pathways* of the vegetative system are more easily determined than the centripetal, if electric stimuli be allowed as a method of delimiting them. There is, however, little agreement con-

cerning the *cortical* representation of internal organs. On the one hand, Bechterew (1911) described in much detail the centers of these various organs and their functions. On the other hand, L. R. Müller (1931) denied such a localization. Allers (1919) believed that neurones whose cell bodies lie in the cortex, synapse in the diencephalon in a manner similar to the synapse found in the thalamus on the centripetal side. If one studies the region of the cortex from which the internal organs in question can be stimulated, it is found that the majority of such foci lie within the motor area or within its vicinity. This is true not only for organs containing smooth muscle (intestine, pelvic viscera, and blood vessels), but also for glands (sweat glands, salivary glands). There is a close topographical relation between the cortical areas from which activities of given vegetative organs can be elicited, and the cortical centers which innervate certain skeletal muscles found peripherally in the vicinity of these organs. For example, difficulties with the innervation of the urinary bladder are observed in injuries of a region near the center for the hip muscles (Czyhlarz and Marburg, 1901). In like manner, bilateral injuries of the lobus paracentralis (center of the foot muscles; Kleist, 1918, and Foerster, 1918) are often accompanied by loss of voluntary control of micturition. This generalization is further substantiated by the ease with which a contraction of the pupil can be produced from areas lying in the neighborhood of the centers from which movements of the eyeballs can be elicited. Localized changes of the vaso-motors of the limbs (vaso-motor form of Jacksonian epilepsy) are observed in diseases of the cortical areas of the limb musculature involved. Salivation in epileptic attacks is produced by injuries near the most ventral part of the gyrus centralis anterior (For details, see Spiegel, 1928).

The cortico-fugal pathways for the vegetative organs appear also to be closely related in position to the pyramidal tract. This is true within the internal capsule, within the peduncle, within the rhombencephalon and within the spinal cord. Lying here in dorsal parts of the lateral column are the centrifugal fibers which carry impulses to the vegetative organs. Recently the writer, in co-operation with Hashimoto, has demonstrated that the accelerator fibers for the heart travel in that region. Further, the writer and MacPherson (1925) have stimulated the peduncle after transverse section of the different

tracts of the spinal cord and observed the contraction of the bladder. Contrary to the deduction of L. R. Müller that the centrifugal pathways for the innervation of the bladder lie in the posterior columns, they found that these pathways are situated in the lateral column. This is in accordance with the results of Stewart (1899), who produced a contraction of the bladder by direct stimulation of the lateral column.

The foci of cortical representation for vegetative organs appear, then, to lie near those of some closely related skeletal muscles, and the descending systems for these organs are topographically related to the pyramidal tract. These conclusions suggest that the cortico-fugal fibers which carry impulses to skeletal muscles, and those which regulate the activity of internal organs, may be partially identical—that is, that the axis-cylinders of the pyramidal tract may stimulate (directly, or by the help of intercalary cells) both the cells of the ventral horn and those of the lateral intermedio-column within the same segments (Fig. 1). In some instances, such as that of the pelvic nerve, the fibers of the pyramidal tract may end only on the vegetative cells of the spinal cord. This general conclusion is supported by the finding that the cortico-fugal fibers which carry impulses to striated muscles and those which stimulate vegetative organs degenerate at the same time. If, after the frontal area and the motor cortex have been removed, the internal capsule be stimulated at different intervals, the movement of the eyeballs and dilatation of the pupil are missed, at the same. Friedberg (working with Spiegel, 1931) observed a similar simultaneous loss of blood pressure response and of movements of skeletal muscles. The pyramidal tract, however, does not seem to be the only way by which centrifugal impulses can reach the internal organs. Cortical impulses may reach the hypothalamic centers by way of fibers from the frontal lobe to the globus pallidus, or to the thalamus—that is, we have to distinguish a pyramidal part and an extra-pyramidal part of the centrifugal vegetative tracts.

The "center" of the latter part is thought to be the *hypothalamus*. The importance of this region for the regulation of vegetative functions is shown by many findings. Krehl (1912) and his co-workers demonstrated that the tuber cinereum plays no mean rôle in the regulation of temperature in homoiothermic animals. Furthermore, Karplus and Kreidl (1909–1928) have observed, after stimulating the

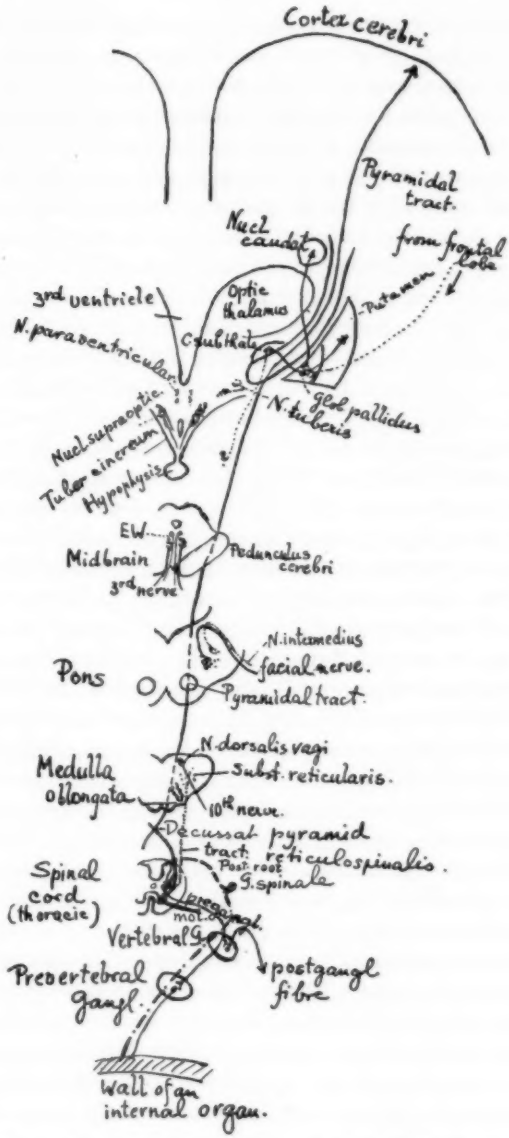

FIG. 1. CENTERS OF THE VEGETATIVE NERVOUS SYSTEM

area of the corpus Luysii, not only contraction of the smooth muscles of the orbit and its blood vessels and of the urinary bladder, but also an increased sweat secretion. These results were also observed after time had been given for the degeneration of the neighboring corticofugal fibers. Symptoms, which were once believed to be only of pituitary origin, such as polyuria, glycosuria, dystrophia adiposogenitalis, are now observed in pathological conditions in man and in animal experimentation also as sequelae of injuries of the tuber cinereum; the pitituary gland in these cases was histologically normal (see Aschner, 1912; Bailey and Bremer, 1921; Camus and Roussy, 1914, 1922; Leschke, 1919; Pick and Molitor, 1925, 1926; Smith, 1927).

Within the hypothalamus, especially in that part known as the tuber cinereum, more or less discrete groups of cells are observed. Of these groups the oldest, phylogenetically speaking, are the nuclei supra-opticus and paraventricularis. The newer group in phylogeny consists of the nuclei tuberis and the nucleus tubero-mamillaris (mamillo-infundibularis, Malone). In some animals, areas of greater density within the central grey arrange themselves into what has been called perifornical nuclei and the nucleus suprachiasmaticus (Spiegel and Zweig, 1919). Some writers have attempted to localize the varied physiological and pathological effects observed after stimulation or injury to one or more of these cell groups, but there is no unanimity (*cf.* the divergent opinions of Camus and Roussy, 1922; Dresel and Lewy, 1922; Greving, 1928), and there is little hope that such endeavors might lead to a definite result; for in the mammals used for experiment most of these nuclei are not sharply delimited and, worse still, their homologies are not definitely settled. This is not the only difficulty present in this region. There is another just as great: the different components of metabolism are so closely related that they influence each other. A pathologically functioning heat regulation may, for example, influence the water metabolism. To the writer it does not seem possible to localize within special groups of cells temperature regulation, carbohydrate, fat or water metabolism. Nevertheless, the tuber cinereum can be divided into two groups of nuclei: first, those innervating the pituitary gland, and second, those sending their axones toward lower centers. To the first group belongs the nucleus supra-opticus, and perhaps parts of the nuclei tuberis and

the nucleus paraventricularis (*cf.* Greving, 1928; Pines, 1925; Stengel, 1926). The second group, including the remainder of the nuclei tuberis and the nucleus mamillo-infundibularis, seem to send their impulses toward lower centers. It is possible to conjecture that the first group exerts an influence upon all those mechanisms which the pituitary gland controls, and that the second group regulates the function of the glands supplied by the splanchnic nerve (liver, adrenal) and perhaps also of some hormonal glands, like the thyroid. It may be in this way that they regulate the metabolic activities and the hydration (water content) of tissue. The nuclei tuberis, which is phylogenetically the youngest of these nuclear groups (Spiegel and Zweig), seems to play a rôle in the mediation of cortical (psychical) impulses to these mechanisms.

From where do the cells of the tuber cinereum receive their impulses? Are they excited only by stimuli reaching them through the blood stream, or also by impulses from higher centers? Theoretically, it is possible for impulses from the strio-pallidum via the pallido-fugal tracts to reach the corpus Luysii and the tuber cinereum. Changes in the state of contraction of the smooth muscles of the pupil, the intestine, the uterus, and the blood vessels, have been observed following stimulation of the striatum. But, since the fibers of the internal capsule pass through the striatum, it is not possible to stimulate the striatum without at the same time, in all probability, exciting the fibers of the internal capsule. Spiegel and Takano (1928) destroyed, unilaterally, the frontal lobe and the motor cortex; after time had been allowed for complete degeneration of the cortico-fugal fibers, the striatum was stimulated bilaterally. Stimulation of the striatum on the operated side did not cause either the pupil or the blood vessels to contract as did stimulation of the striatum on the normal side. The results of Schüller (1902) substantiate these findings. He found that the movements of skeletal muscles and the system of respiration could not be modified by stimulation of the striatum in which the internal capsule had been allowed to degenerate. Neither could Spiegel and Takano observe contraction of the urinary bladder under like conditions when that organ could no longer be compressed externally by the contraction of the abdominal musculature. The only conclusion from these experiments is that the contractions of smooth

muscle organs following stimulation of the striatum is due mainly, not to a stimulation of the cells of the striatum but to the corticofugal tracts passing through it.

Nevertheless, this result does not mean that the striatum is not able to affect the lower vegetative motor centers. What is the possibility of its exerting an influence upon metabolism? Richet's (1884), and Aronson's and Sach's (1885) reports that they produced fever by a puncture of the nucleus caudatus might be construed to mean that this part of the brain has some relation to the function of certain glands, or to forms of metabolism in the striated muscles. Since it is recognized that the tuber cinereum controls not only temperature but also varied forms of metabolism and is closely connected with the striatum, it seemed possible that the latter ganglion might influence different forms of metabolism through those fiber relationships. Spiegel and Reynolds (1930) studied the changes in diuresis following puncture of the head of the nucleus caudatus, comparing such changes with those found in the temperature of the body. This operative procedure resulted not only in fever but also in polyuria, as well as an increase in the specific gravity of the urine. Tokay, working with Spiegel, (1931) studied the sodium content of the urine after this puncture and found a decided increase in the secretion of NaCl. This increase in urine excretion was most marked in those animals which showed a definite increase in body temperature, although the polyuria often lasted a few days longer than the puncture fever. Simple injury to the cortical substance, or to the caudal part of the lateral ventricle, was not followed by such a polyuria. Only those lesions that involved the anterior horn of the lateral ventricle, or the cortex and its underlying fibers close to the wall of the ventricle, gave these results (Spiegel and Reynolds). Thus it seems that the anterior part of the striated body affects in some manner the water-salt balance of the body, perhaps through its fiber relation with the tuber cinereum.

Now, how do the impulses from the tuber cinereum reach lower centers? Greving (1928) described tracts which originate in the medial part of the central grey substance, in the substantia reticularis of the hypothalamus, and in the nuclei tuberis; their exact terminations are unknown. The nucleus tubero-mamillaris (mamilloinfundibularis) is probably connected with the corpus mamillare, and

it is known that this nucleus is the origin of fiber systems (fascic. mamillo-tegmentalis, pedunculus corporis mamillaris), to the subst. reticularis and to the central grey substance of the midbrain; their exact terminations, however, remain unknown. Also, the relation of the substantia reticularis of the rhombencephalon to higher centers is not mapped. We do not know which part of the descending tracts ends in the pons and in the medulla oblongata, and which part goes directly toward the spinal cord.

Many vegetative centers have been postulated and circumscribed by stimulation within the *rhombencephalon*, especially the subst. reticularis. The change in the salt concentration of the urine after puncture of the floor of the fourth ventricle was considered to be the consequence of the stimulation of the cells within this area of injury, but this effect has not been analyzed as to whether it is due to a stimulation of fibers from higher centers, or locally of the substantia reticularis. Müller and Glaser even doubt the existence of a general vasomotor center in the rhombencephalon, but we know that pressor reflexes (Karplus and Kreidl, 1918) and depressor reflexes due to the stimulation of the labyrinth (Spiegel and Démétriades, 1924) or of the depressor nerve (Spiegel and Yaskin, 1928) persist even after total transverse section of the midbrain. Ranson and Billingsley (1916) have shown that a fall of blood pressure can be produced by faradic stimulation of the floor of the fourth ventricle within the area postrema. Further studies seem necessary to analyze how far this effect is due to the stimulation of the centripetal part of depressor reflex arcs (central endings of the vestibular, 9th or 10th nerve) and how far it is due to the stimulation of a general vasodilator center.

Vegetative centers are found, therefore, in many levels of the central nervous system and not only in the segmental cells of origin of preganglionic fibers (vegetative parts of cranial nuclei, vegetative nuclei in the thoracic, upper lumbar and sacral segments). The activity of these cell groups is modified by that of the formatio reticularis of the rhombencephalon, of the hypothalamus, and of the forebrain. The question arises: what does this manifold distribution of "centers" for the control of vegetative organs and their function mean? Starting from this question, the innervation of the blood vessels was studied by Spiegel and Yaskin (1928). The influence of the destruction of

the different parts of the central nervous system upon the level of the blood pressure and the excitability of the remaining parts of the central vasomotor mechanisms were studied. The extirpation of the cortex, or of the whole forebrain, gave no marked effect. Apparently, there was also no sure change of blood pressure when the thalamus was removed. But when a transverse section was made through the caudal part of the midbrain or posterior to it, the increase of blood pressure observed endured for ten minutes. This maintenance of pressor effect was independent of decerebrate rigidity. This rise of blood pressure was due, in part to the loss of inhibitory impulses from higher centers, and in part to the mechanical stimulation of the operation. The rôle played by mechanical stimulation was demonstrated by the finding that, after the initial rise in blood pressure had subsided, a second puncture in the cranial part of the pons produced a second rise, weaker and shorter in duration than the first. The loss of inhibitory impulses was shown by the fact that, after section of the midbrain, the reaction to various pressor stimuli—such as the stimulation of the centripetal fibers of the sciatic nerve, asphyxia, and intra-ventricular injection of concentrated NaCl solution—was stronger than in the intact animal, or in one following the removal of the cortex and the thalamus. These results should be considered in any study of the influence of toxins upon blood pressure in decerebrate animals. In the latter preparation, the rise of blood pressure following injection of toxins is greater than normal, as a result of the increased excitability of the remaining vaso-motor centers.

If one compares the function of the different parts of the central vaso-motor apparatus, it seems that the segmental centers are simply the place of origin of the pre-ganglionic centrifugal fibers, and as such are mainly concerned in segmental reflexes. The rhombencephalic part of the apparatus rules the blood pressure as a whole, especially the vessels in the area of the splanchnic nerve. Concerning the diencephalic centers, the experiments of Schrottenbach (1914, 1916), especially, led to the conclusion that the hypothalamus plays an important rôle in the genesis of vasomotor symptoms accompanying psychic excitation. This is in agreement with the assumptions of Czyhlarz and Marburg (1901) that the thalamus has to do with psychoreflexes upon internal organs like the bladder, and the findings of Karplus and

Kreidl that transverse sections of the midbrain abolish the dilatation of the pupil that follows painful stimulation. These relations of the hypothalamus to vegetative symptoms due to psychic excitation might have to do with the fact that mechanisms producing a primitive form of consciousness are localized in the diencephalon. While changes between sleep and wakefulness have been observed in animals without forebrain (Goltz, 1892; Rothmann, 1923; Karplus and Kreidl, 1914; Schaltenbrand and Cobb, 1931) one can produce a sleep-like state lasting several weeks by a double-sided injury of the thalamus (Spiegel and Inaba, 1927).

But the hypothalamus, especially the tuber cinereum, is important also for other functions that are quite independent of psychical impulses, for instance, temperature regulation. The co-ordination of the different parts of metabolism seems to be one of the important functions of the hypothalamus. The influence of the cortex is not limited to the voluntary innervation of organs with smooth muscles, a type of innervation that has been only rarely observed. It seems that cortical centers also play a rôle in the vegetative reactions that follow, for instance, painful stimuli. This is shown by the fact that the dilatation of the pupil after painful stimuli is less marked in a decorticated than in a normal animal (Amsler, 1924). In other words, the pain impulse which causes the dilatation of the pupil takes its way partly through the hypothalamus, partly through the cortex.

The higher centers seem to exercise a control upon the vegetative "final common pathway" in a manner similar to that described by Hering and Sherrington (1898) for the somatic—the reciprocal innervation of the agonist and antagonist. This is true, not only for reflexes that have their center in the medulla oblongata like the depressor reflex (Bayliss, 1893, 1908; Asher, 1908, 1918; Brücke, 1912, 1922), but also for reactions of the hypothalamus, as the author's own experiments in collaboration with Dr. Hashimoto have shown. The increase of the pulse rate following painful stimuli persists after severance of both vagi, but it is weaker than before, because the inhibition of the vagus plays a rôle in this mechanism, besides the stimulation of the nervus accelerator. The action of this law can be seen in cortical reactions too, as experiments of Braunstein (1895) on the pupil have shown. The loss of the light reflex during an epileptic fit

can be explained by the same mechanism (stimulation of the cervical sympathetic, and inhibition of the oculomotor nucleus by the cortical excitation).

One might say that in general all increase of psychic activity is accompanied by an increase of the excitation in the sympathetic part of the vegetative system and the inhibition, at least, of some parts of the parasympathetic (*cf.* Cannon, 1920; Metzner, 1913; Weinberg, 1924). On the other hand, during sleep the increase in tonicity of the parasympathetic part of the vegetative system is accompanied by the depression of the sympathetic part, illustrated by miosis of the pupil, bradycardia and lowered blood pressure. One has here to do with a further analogy between the vegetative and somatic mechanisms; namely, that just as with successive spinal induction (Sherrington, 1906) the reciprocal inhibition of antagonists is followed by their increased excitation and inhibition of the former agonists ("Successive central induction").

These are only the outlines of some of the probable means of regulation of the activity of the "final common pathway" by the more cephalward areas of the central nervous systems. That the understanding of their relational physiology and anatomy has not even completed its period of gestation is clear. Nevertheless, it perhaps demonstrates that a great deal of experimental work has been undertaken and that the older definition of the vegetative nervous system as composed only of two efferent neurones, a preganglionic and a postganglionic, is no longer held. Not only does the autonomic nervous system consist of afferents and efferents distributed as peripheral nerves, but also there exists a complicated adjustor apparatus centrally, which may yield to further attempts of analysis.

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EXPERIMENTAL YAWS[1]

I. Comparison of the Availability of the Rabbit and Monkey for the Isolation of Strains of Yaws

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The question of the relationship of yaws to syphilis has been for years a fruitful source of speculation and is still under discussion. The problem has been approached from several angles of which one has been that afforded by the experimental method. The present communication is the first of a series dealing with experiments designed to throw light upon the question whether or not yaws and syphilis are identical diseases. The experiments that will be reported in subsequent papers record attempts to determine whether yaws, experimentally induced, is similar to experimental syphilis, and whether there is any immunological relationship between strains of spirochetes recovered from yaws patients, on the one hand, and from syphilitic patients, on the other. Previous observers have made similar comparisons of these experimental infections but not, so far as we are aware, with strains of yaws and syphilis spirochetes recovered in the same locality. This seems to us an important point and for that reason we sought to obtain such strains from persons in the same country and belonging to the same race.

Although methods for establishing a syphilitic infection in experimental animals were well known, a review of the literature prior to undertaking the present study on the subject of the transfer of yaws from man to animals afforded the authors little information as to how this could best be accomplished. In this paper therefore we wish first to record, for the benefit of others, our experiences in transferring yaws virus to two types of laboratory animals, rabbits and monkeys, and secondly, to establish for future reference the identity of several strains of yaws and one strain of syphilis virus, which we have isolated. In

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brief, transfer of yaws virus from patients to rabbits or monkeys was attempted in ten instances with a successful issue in eight. In our hands the rabbit proved to be a more favorable animal than the *Macacus rhesus* monkey for the isolation of *T. pertenue* and intratesticular inoculation of the rabbit was found to be superior to the intracutaneous method or inoculation upon the surface of a granulating wound.

HISTORICAL

In 1906 monkeys were successfully infected for the first time with yaws by Neisser, Baerman and Halberstadter (1), and in rapid succession successful transfers from human beings to monkeys and the higher apes were reported by Castellani (2), Ashburn and Craig (3) Halberstadter (4) and Nattan-Larrier (5). In more recent years Schobl (6) has made a number of such transfers and has mentioned unpublished instances of transfers by others (Strong and Guererоro, Walker).

The rabbit was first successfully infected by Nichols (7) in 1910 with virus from an infected monkey, but it was not until 1920 that transfer of the disease from a human being to the rabbit was accomplished by the same worker (8). Ikegami (9) reported two instances of successful transfer from patients to rabbits, while only recently Matsumoto published in his monograph "Experimental Syphilis and Framboesia" (10) the results of experiments performed by his associates Ikegami, Takasaki, and Hashiguchi.

On reading these reports one is led to the conclusion that the transfer of yaws from human beings to rabbits and monkeys is not always easy of accomplishment.

NATURE AND SOURCE OF MATERIAL

The original transfers from patients here reported were made in Haiti. Infectious forms of yaws are encountered in that country, as elsewhere, almost exclusively among the inhabitants of rural communities and, as it was deemed important to obtain strains of yaws spirochetes from widely separated districts, it was frequently necessary to perform the transfer of virus under field conditions. The details of an experiment, therefore, were often modified by the exigencies of the time and place.

The patients from whom virus was collected were all full-blooded negroes and only those presenting classical lesions of early yaws were selected. As detailed a history as possible was obtained and a physical examination was made by one of the authors. Photographs of the lesions were taken and in each instance *Treponema pertenue*[2] was demonstrated by dark-field examination of material from one or more of the lesions. Serologic examination of the blood was made when practicable.

All of the original transfers were made in Haiti between November 17, 1929 and February 5, 1930, while some of the subsequent studies were carried out in the laboratories of the Johns Hopkins University.

COLLECTION OF MATERIAL

The technique employed for the collection of virus from patients was the same in each case. Virus was obtained invariably from early skin lesions of the disease and not infrequently more than one lesion was used. Those framboesiform lesions which were covered with a crust were found, upon removal of the crust, to yield treponemes in great abundance. As a rule, material was obtained from lesions on the upper portions of the body in order to minimize as far as possible the chances of secondary infection. The lesion having been selected, the crust and surrounding skin was cleansed with 70 per cent alcohol and the crust covering the lesion moistened with normal salt solution. This crust was then removed, exposing a pink granular surface which had somewhat the appearance and consistency of the dorsum of the human tongue except that the corrugations were much larger and coarser than those of the tongue. The surface of the lesion was often covered by a thin film of whitish secretion which on microscopic examination proved to consist mainly of dead and degenerated leucocytes, and while this material was often rich in *T. pertenue* it was, with one exception noted below, wiped away and discarded. The surface of the lesion was then irritated by friction or excoriation and pressure applied on the base until a small amount of serum exuded. To facili-

[2] No attempt has been made to differentiate on a morphological basis *T. pallidum* from *T. pertenue*. The use of these terms is based upon the clinical diagnosis of the case from which the organism was recovered. The differentiation between these and other spirochetes, however, is clear.

tate collection of the serum it was emulsified in one or two drops of normal salt solution, drawn up in a capillary pipette and placed in a small receptacle until a sufficient amount had been obtained for one inoculation. Actively motile *T. pertenuia* were usually abundant in this material and no other spirochetes were visible. It was found that the treponemes tended to lose their motility rather rapidly, so that it was thought expedient to make each inoculation as soon as a sufficient quantity had been collected. The amount each animal received, therefore, varied to some extent, although an effort was made to have each inoculum of approximately the same strength. The entire procedure was repeatedly checked by dark-field examination of the material for the number, morphology and activity of the treponemes.[3] Strict asepsis was maintained throughout.

METHOD OF INOCULATION

Rabbits and monkeys were the laboratory animals used in the investigation. Among the former were represented most of the common laboratory breeds while the monkeys were young male animals of the *Macacus rhesus* variety. All animals were secured in the United States and transported to Haiti.

Monkeys were not employed in every experiment but when used they were invariably inoculated over each eyebrow by scarification, and intracutaneously over each deltoid region. In inoculating rabbits three methods were commonly employed, the intratesticular, the intracutaneous and the deposition of virus on the surface of a granulating wound. In the first method the virus was deposited into the substance of the testis through a small needle. Frequently both testes were inoculated but at times only one. Intracutaneous inoculation was commonly made in two areas on the rabbit's back, from which the hair had been removed with a mixture, the chief ingredient of which was barium sulphide. Inoculation of a granulating wound was made according to the method described by Chesney and Kemp (10) for experimental syphilis. The age of the wound at the time of inoculation varied in different experiments from the fact that it was

[3] Attention might be called here to the fact that the sun affords admirable illumination for the dark-field microscope. In the tropics, such a source of illumination is usually both convenient and dependable.

impossible to predict the day on which a patient suitable for transfer of virus might be encountered. In the earlier experiments a single method of inoculation was employed for each rabbit, but as the investigation proceeded, the limited supply of animals made it necessary frequently to inoculate a rabbit in more than one way.

Transfer of yaws spirochetes from eleven patients was performed, although the animals inoculated in experiment X were discarded because of a questionable feature in the case from which the virus was obtained.

In addition to the transfer of virus from patients with yaws, material was transferred from a patient with a classical primary syphilitic genital lesion. The details of this transfer will not be dwelt upon, since the procedure is well known and no difficulty was experienced in producing a successful result in rabbits.

RESULTS

The important data on these experiments are given in table 1. A total of 34 rabbits were inoculated in one or both testes with material containing actively motile *Treponema pertenue*. Of these 14 subsequently developed lesions of yaws at the site of inoculation, the incubation period ranging from a minimum of 33 days to a maximum of 108 days. Of the remaining 20 rabbits, 6 developed secondary infections causing them to die or to be discarded within a few days after inoculation, 6 more, for one reason or another, were observed for periods less than 90 days but remained negative during this time, and 8 remained negative for 130 days or longer.

Twelve rabbits were inoculated intracutaneously on the back but in not one was a definite lesion produced, although in 3 a questionable lesion developed at the site of inoculation. Eight of the 12 rabbits were observed for at least 120 days, 3 for a somewhat shorter period, and one died on the 6th day after inoculation. In 2 animals simultaneous inoculation in the testis gave rise to a definite testicular lesion, while the back remained unaffected.

Inoculation of virus upon the surface of a granulating wound was carried out in 13 instances, the age of the wound at the time of inoculation varying from 1 to 23 days. In 7 rabbits the period of observation was 128 days or longer; 4 died within a week after inoculation, and in

TABLE 1

EXPERIMENT NUMBER	PERTINENT DATA CONCERNING PATIENT	ANIMALS INOCULATED*	METHOD OF INOCULATION	RESULT OF INOCULATION		INCUBATION PERIOD	DURATION OF OBSERVATION	REMARKS
				Local lesion	Dark-field			
						days	*days*	
I	E. S., male, 14 yrs. Residence, Leogane. Virus obtained from lesion on face	2706	Wound—1 day	?	0	–	155	Second generation positive. Designated Strain Y 1
		2710	Wound—1 day	?	0	–	155	
		2711	Intracutaneous	0	–	–	123	
		2712	Intracutaneous	0	–	–	155	
		2713	Testis—right	0	0	–	73	
		2714	Testis—right	+	+	108	–	
II	M. D., male, 39 yrs. Residence, St. Michel. Virus obtained from lesion on forehead	2708	Wound—4 days	?	–	–	150	Strain not recovered
		2717	Intracutaneous	0	–	–	150	
		2719	Intracutaneous	?	0	–	179	
		2720	Testis—right	Abscess	0	–	153	
		2721	Testis—right	0	0	–	153	
		Ape 1	Eyebrows	0	–	–		
			Deltoids	?	0	–	164	
III	E. D., female, 12 yrs. Residence, Petit Goave. Virus obtained from lesion on chin	2722	Intracutaneous	?	0	–	375	Second generation positive. Designated Strain Y 3
		2723	Intracutaneous	?	0	–	130	
		2724	Testis—right	+	+	51	–	
		2730	Testis—right	+	+	33	–	
IV	D. D., male, 2 yrs. Residence, Limbe. Virus obtained from lesion on back	2718	Wound—14 days	–	–	–	2	Second generation positive. Designated Strain Y 4
		2729	Wound—14 days	–	–	–	2	
		2734	Intracutaneous	0	–	–	129	

		2738	Intracutaneous	0	–	–	129	
		2735	Testis—Right	+	+	93	–	
		2736	Testis—right	0	–	–	131	
		2737	Testis—right	Abscess	–	–	47	
		Ape 2	Eyebrows	0	–	–	215	
			Deltoid	?	–	–		
V	N. T., male, 9 mos. Residence, Grande Rivière. Virus obtained from lesion on forearm	2728	Wound—17 days	?	0	–	128	Second generation positive. Designated Strain Y 5
		2731	Wound—17 days	0	–	–	128	
		2739	Testis—right	0	0	–	46	
		2740	Testis—right	0	–	–	160	
		2741	Testis—right	+	+	62	–	
		2742	Testis—right	+	+	62	–	
VI	A. P., male, 33 yrs. Residence, Mirebalais. Virus obtained from lesion on back	2744	Wound—10 days	0	–	–	140	Second generation positive. Designated Strain Y 6
			Testis—right	?	–	–		
		2747	Wound—10 days	0	–	–	140	
			Testis—right	Abscess	–	–		
		2753	Intracutaneous	0	–	–	84	
			Testis—right	+	+	52		
		2754	Intracutaneous	–	–	–	6	
			Testis—right	–	–	–		
		Ape 3	Eyebrows	+	0	80	197	
			Deltoids	?	0	–		
VII	M. P., male, 39 yrs. Residence, Mirebalais. Virus obtained from lesion on forearm	2755	Testis—right	+	+	44	–	Second generation positive. Designated Strain Y 7
		2756	Testis—right	Abscess	0	–	129	
		2757	Testis—right	?	–	–	58	
		2758	Testis—right	?	–	–	129	

* In the column "Animal Inoculated" individual rabbits are designated by the higher numbers; monkeys are designated Ape 1, etc.

TABLE 1—*Concluded*

EXPERIMENT NUMBER	PERTINENT DATA CONCERNING PATIENT	ANIMALS INOCULATED*	METHOD OF INOCULATION	RESULT OF INOCULATION		INCUBATION PERIOD	DURATION OF OBSERVATION	REMARKS
				Local lesion	Dark-field			
						days	*days*	
VIII	H. P., female, 53 yrs. Residence, Leogane. Virus obtained from lesion on face	2743	Wound—23 days	–	–	–	2	Strain not recovered
			Testis—right	Abscess	–	–		
		2746	Wound—23 days	–	–	–	7	
			Testis—right	Abscess	–	–		
		2727	Testis—right	Abscess	–	–	11	
		2761	Testis—right	Abscess	–	–	11	
		2762	Testis—right	Abscess	–	–	1	
		Ape 4	Eyebrows	?	0	–	184	
			Deltoids	+	0	34		
IX	E. B., female, 8 mos. Residence, Gressier. Virus obtained from lesion on forearm	2763	Wound—2 days	0	–	–	69	Second generation positive. Designated Strain Y 9
			Testis—right	+	+	36		
		2767	Wound—2 days	0	–	–	70	
			Testis—right	+	+	41		
		2716	Intracutaneous	0	–	–	70	
			Testis—both	?	0	–		
		2768	Intracutaneous	0	–	–	70	
			Testis—both	+	–	40		
		Ape 5	Eyebrows	+	0	120	180	
			Deltoids	?	0	–		
XI	J. D., male, 8 yrs. Residence, Cafond. Virus obtained from lesion on forearm	2770	Testis—right	Abscess	–	–	75	Second generation positive. Designated Strain Y 11
		2771	Testis—right	+	–	60	–	
		2772	Testis—both	+	–	60	–	
		2773	Testis—both	+	+	39	–	

2 the period of observation was 70 days. No rabbit developed a definite lesion at the site of inoculation. In 4 animals suspicious areas appeared in the healing wound, or else healing was thought to be retarded, but dark-field examination of material from these areas was always negative. In 2 animals in which testicular inoculation also was made lesions appeared in the testis but not in the traumatized area on the back.

It seems clear, then, that in these transfers to rabbits intratesticular inoculation was by far the most satisfactory of the three methods employed. One of the obstacles to the success of a testicular inoculation was a non-specific reaction brought about by some unknown agent in the inoculum, perhaps bacteria or degenerated tissue. In experiment VIII, for example, the inoculum was composed largely of the thick secretion lying just under the crust of the human lesion; the day following inoculation each inoculated testis was greatly swollen, there was edema of the scrotum and within a few days the animal either succumbed or the entire testis became necrotic. In 5 other rabbits, in which no yaws lesion could be demonstrated there occurred mild reactions in the inoculated testis which served to confuse the observer.

During the course of the investigation 5 monkeys were inoculated with yaws virus from as many patients, each animal being inoculated intradermally by scarification over each eyebrow and intracutaneously over each deltoid muscle. The results of these transfers were disappointing, for although definite lesions were produced in two, and questionable lesions in the other 3 animals, the lesions were usually insignificant and *T. pertenue* was never recovered despite repeated dark-field examination of serum and scrapings from the lesions.

Ape no. 1 developed a few scaly macules over one inoculated area 104 days after inoculation while the other areas remained negative. It will be noted, however, that the rabbits inoculated with this virus failed to show definite lesions and the strain was not recovered. Ape no. 2 developed a superficial lesion with annular configuration in one area (deltoid) 126 days after inoculation, but this disappeared within a week and the animal remained negative although the strain was recovered from a rabbit. Ape no. 3 developed definite crusted papules in each eyebrow 80 days after inoculation. Upon removal of the crust there was revealed an ulcerated papule which bled easily, and

it was felt that the lesion resembled those described by other investigators. The lesions became larger and persisted for about two months but never during this time could *T. pertenue* be demonstrated by dark-field examination in spite of repeated attempts. One of the lesions was excised and emulsified, but the emulsion showed no treponemes and upon inoculation into the testes of 2 rabbits failed to produce the disease in them. A rabbit inoculated with virus from the same patient developed in 52 days a lesion from which the strain was recovered. Ape no. 4 exhibited a group of tiny horny papules over each deltoid region 35 days after inoculation. The lesions grew larger but despite the rather typical appearance of the lesion no *T. pertenue* could be demonstrated, and an emulsion of one of the lesions failed to produce disease upon inoculation into rabbits. The lesions persisted for approximately four months and the blood Wassermann test became positive. The rabbits inoculated with virus from this patient died within a few days after inoculation, from secondary infection, although in the monkey the incubation period of 35 days would seem to exclude secondary infection as a cause of the lesion. Ape no. 5 developed insignificant lesions in each inoculated area 40 days after inoculation, but again no spirochetes could be demonstrated by dark-field examination or rabbit inoculation. Virus from this patient produced lesions when inoculated into rabbits.

From the foregoing, granting that some of the lesions observed in the monkeys were probably those of yaws, it seems obvious that they were quite inadequate as a source for the perpetuation of strains.

The results of these experiments may be summarized in the following manner. In a parallel series of inoculations in which yaws virus obtained from patients was introduced into rabbits and monkeys, 8 out of a possible maximum of 10 strains were isolated and carried successfully through a second generation of rabbits. In each instance the strain was recovered through intratesticular inoculation of rabbits, whereas in no instance could it be recovered from the monkey or through intracutaneous or granulating-wound inoculation of rabbits. It is realized that the number of monkeys inoculated was small; moreover, it is difficult to reconcile the disappointing results obtained in them with the work of some of the earlier observers. Nevertheless, this comparison between the availability of the monkey and rabbit for

the isolation of *T. pertenue* appears to be a reasonable one, for the two species were inoculated under similar conditions with approximately equal amounts of the same virus. Aside from the difference in the number of positive results, the rabbit on account of economic factors, ease of handling and time saved, is the more satisfactory animal for purposes of inoculation. The point is stressed, not alone from the standpoint of the isolation of strains of yaws spirochetes, for after all the necessity for such presents itself infrequently, but because it would seem to apply as well to any investigation demanding the transfer of tissue from man to animals in the search for *T. pertenue*.

SUMMARY AND CONCLUSIONS

1. Yaws virus from native Haitians suffering with early yaws was transferred to both rabbits and monkeys. In all, material from 10 different patients was transferred. Eight strains of yaws spirochetes were recovered by inoculating rabbits and propagated through a second generation of this species.

2. Five *Macacus rhesus* monkeys were inoculated with material from as many different patients, but, although lesions were produced in some, *T. pertenue* could not be demonstrated, and there was no assurance, therefore, that the organisms were transmitted to these animals.

3. Rabbits inoculated by three methods—testicular, intracutaneous, and granulating wound—developed definite yaws lesions only after testicular inoculation.

4. Of 34 rabbits inoculated in one or both testes, 14 developed definite yaws lesions.

5. A strain of syphilis spirochetes was successfully transferred from a native Haitian to rabbits.

6. For the transfer of yaws from man to animals, the rabbit in our hands has proved to be a more suitable experimental animal than the *Macacus rhesus* monkey. Only testicular inoculation of the rabbit yielded satisfactory results.

APPENDIX

Abstracts of clinical data of patients from whom transfers were made

Experiment I. E. S., male, aged 14 years. Residence, District of Leogane. "Mother yaw" appeared on left knee in February, 1929, and in August numerous

lesions appeared on the face. There had been no constitutional symptoms. The physical examination was negative except for the presence of numerous characteristic lesions of early yaws on the face, neck, scalp, forearms and back. There was a healing yaw on the left knee. Material collected from a lesion on the face showed many actively motile *T. pertenuia*. The Kahn test on the blood was positive.

Experiment II. M. D., male, aged 39 years. Residence, District of St. Michel. A lesion had appeared in the submental region 15 days previously and a few days later yaws had appeared on the face, arms, scalp and chest. These lesions were characteristic of early yaws. Save for general glandular enlargement the remainder of the physical examination was negative. Material for inoculation, which showed many actively motile *T. pertenuia*, was obtained from a lesion on the forehead. The Kahn test on the blood was positive.

Experiment III. E. D., female, aged 12 years. Residence, District of Petit Goave. One month previously a lesion had appeared on the chin and a few days later lesions appeared elsewhere on the face, on the left knee and in the palms of each hand. There was a large painless lymph node in the submental triangle but no other glandular enlargement. The general physical examination showed only moderate undernutrition. The skin lesions were characteristic of early yaws. Material for inoculation obtained from a lesion on the chin, showed numerous actively motile *T. pertenuia*.

Experiment IV. D. D., male, aged 2 years. Residence, District of Limbe. Scattered over the face, trunk and extremities were typical framboesiform lesions (fig. 1). These had been present for about 5 months. There was a marked general glandular enlargement, but the remainder of the physical examination was negative. Virus obtained from a lesion on the back, showed many actively motile *T. pertenuia*.

Experiment V. N. T., male, aged 9 months, Residence, Grande Rivière. The child was born at term and had been well until skin lesions developed four months prior to admission to the clinic. On examination there were many typical lesions over the trunk and extremities, and a few lesions on the palms (fig. 2). There was a general glandular enlargement but nothing else of note in the physical examination. Virus obtained from lesions on the forearm, showed many actively motile *T. pertenuia*.

Experiment VI. A. P., male, aged 33 years, Residence, vicinity of Mirebalais. The past history was non-contributory. Skin lesions had developed on the face, trunk, and genitalia 6 weeks previously. These lesions were found to be typically framboesiform. The general physical examination was negative except for a

FIG. 2

FIG. 1

general glandular enlargement. Material for inoculation was obtained from two lesions on the back. Actively motile *T. pertenuia* were easily demonstrable.

Experiment VII. M. P., male, aged 39 years. Residence, Mirebalais. Nine months previously a mother yaw had appeared on the dorsum of the right foot, and four months later, after the primary lesion had healed, lesions had developed on the face, trunk, and extremities. Aside from slight arthralgia accompanying the skin eruption his general health had been good. The scar of the primary lesion was

FIG. 3

visible; there were many characteristic yaws lesions on the face, trunk, and extremities, some being annular in configuration, and there was a well marked general glandular enlargement. The general physical examination was negative. Serum from a lesion on the forearm showed many actively motile *T. pertenuia*.

Experiment VIII. H. P., female, aged 53 years, Residence, Vicinity of Leogane. Her health had been quite good until the onset of yaws in September, 1929. A lesion on the left leg was the first to appear, and two months later lesions devel-

oped on the face. There had been vague pains in the back and legs, with general malaise. On examination the lesions, which were characteristic of early yaws, were confined to the face (fig. 3), although on the left leg was a scar which was probably the remains of the primary lesion. Except for a general glandular enlargement, the general physical examination was negative. Upon removal of the crusts from the lesions a large amount of thick white material was exposed and under this lay the pink framboesiform surface. This whitish secretion contained *T. pertenuia*

FIG. 4

in great numbers, but inoculation of this material into rabbits was followed by death of the animal in a few days, or else by the formation of large sloughing areas at the site of inoculation.

Experiment IX. E. B., female, aged 8 months, Residence, Vicinity of Gressier. The mother had had active yaws at the time of the baby's birth and even when seen in the clinic there were several lesions which had not yet healed. The baby had

had yaws for about 3 months, although it was not known which lesion was the first to appear. Large crusted lesions covered the forearms and face, and lesions were also present on the thighs and about the genitalia (fig. 4). Removal of the crust from some of these lesions revealed a typical framboesiform surface, and serum from the lesions showed many actively motile *T. pertenuia*. Virus was obtained from a lesion on the forearm.

Experiment X. This man, a native of Croix de Bonquet, exhibited numerous large flat papules on the thighs, genitalia and buttocks. While it was thought that the lesions were yaws, the possibility that syphilis might produce such a picture was recognised and therefore animals to which virus had been transferred were discarded.

Experiment XI. J. D., male, aged 8 years, lived in the mountains about one day's journey from Port au Prince. Four months previously, the patient had developed a "mother yaw" on the right knee and 1 month later yaws lesions appeared on the face, arms, and genitalia. There was a general glandular enlargement, otherwise the physical examination was negative. Virus containing many actively motile *T. pertenuia* was collected from a lesion on the left forearm. The patient was accompanied to the clinic by his sister, age 10 years, who also had early yaws.

The isolation of a strain of syphilis. L. C., aged 35 years, a native of the southern part of Haiti had come to Port au Prince to live 4 years previously. The past history was non-contributory. Fifteen days prior to admission and about 1 week after sexual intercourse a small papule appeared on the glans penis. This grew larger till at the time of examination it presented the appearance of a characteristic primary syphilitic lesion. The chancre was fairly well circumscribed, markedly indurated, involving contiguous portions of the glans, coronal sulcus and prepuce just to the right of the midline, and a portion of its surface was ulcerated. It was neither painful nor very sensitive to touch. Serum expressed from the lesion showed an occasional actively motile *T. pallidum*. In the right inguinal region were two large, discrete, rubbery lymph nodes which were quite characteristic of satellite buboes, but none of the other groups of superficial nodes was enlarged. There were no secondary manifestations and the general physical examination was negative. The Kahn test was positive.

Serum from the chancre was inoculated into one testis of 3 rabbits. One of the large inguinal lymph nodes was excised under local anesthesia, an emulsion was prepared and inoculations were made into the opposite testis of the foregoing rabbits and into both testes of 3 other rabbits. *T. pallidum* could not be demonstrated in this emulsion. A syphilitic orchitis developed in all of the testes of the 6 rabbits inoculated. The incubation period of the lesions varied from 15 to 48 48 days. This strain of *Treponema pallidum* has been designated "Strain K." The strain was carried successfully to a second generation of rabbits.

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SEROLOGICAL REACTIONS WITH HEMOLYTIC STREPTOCOCCI IN ACUTE BACTERIAL INFECTIONS[1]

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The observations presented in this communication concern the agglutination of certain strains of *Streptococcus hemolyticus* by sera derived from patients acutely ill with a variety of acute bacterial infections. In the case of each patient from whom serum was obtained a bacteriological diagnosis was definitely determined. In many instances the illness was proved to be due to organisms other than hemolytic streptococci. By using only those cases in which the infecting bacteria were identified, it was possible to know with certainty whether any relationship existed between the test organism (hemolytic streptococcus) and etiological agents. In addition to the question of serological specificity, the investigation has been extended to include a study of the underlying mechanism of the phenomenon; the results of this, as yet incomplete, analysis are included in this report.

In carrying out the tests, observations were made which demonstrate certain unique characters of the serological reactions. As will be pointed out, evidence has been obtained indicating that the phenomenon is not due to the presence in serum of a "true" antibacterial antibody. In spite of this, a mixture of suitable streptococci with reactive sera results in the clumping of organisms into moderately large masses which fall to the bottom of the tube. The reaction is definite and in gross appearance cannot be differentiated from large flakes of agglutinated bacteria.

When organisms and strongly reactive sera are mixed and shaken, increasing granulation is often visible in 1 to 2 minutes; within 5 to 10 minutes coarse granulation may be readily seen. Optimal reactions are obtained after 1 to 2 hours' incubation in the water-bath at 37.5°C. At the end of this time coarse clumps have collected at the bottom of

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the tube leaving a clear supernatant fluid. When the tubes are shaken the flakes have the appearance of being light and fluffy, but will withstand moderately vigorous agitation. Sera which do not react so markedly, and high dilutions of strongly reactive sera, give the usual gradations in the degree of clumping. In the observations recorded in this report, final readings were made after the tubes had remained in the ice-box overnight following incubation in the water-bath at 37.5°C. for 2 hours.

The capacity of patients' sera to agglutinate hemolytic streptococci has been tested in a wide variety of diseased conditions. The observations reported in this communication are limited to reactions obtained with serum from cases in which the diagnosis was proved both clinically and by laboratory methods. In table I, a representative group of cases of acute bacterial infection is presented which demonstrates the absence of specificity of the reaction.

The list comprises instances of infection not only with *Streptococcus hemolyticus*, but also with *Streptococcus viridans*, *Pneumococcus*, *Staphylococcus aureus*, *Meningococcus*, *Gonococcus*, *B. typhosus*, *B. tuberculosis*, and *Br. melitensis*. From the table it may be seen that agglutination of hemolytic streptococci is demonstrable in high titre regardless of the genus of bacterium causing the disease. Up to the present time, sera have been tested which have been derived from 10 cases of pneumococcus pneumonia, 8 cases of typhoid fever, 2 cases of meningococcus meningitis, 2 cases of acute gonococcal arthritis, 3 cases of lung abscess (no hemolytic streptococci demonstrable in the pus), 3 cases of active pulmonary tuberculosis, 2 cases of undulant fever, 3 cases of subacute bacterial endocarditis (*Streptococcus viridans*), and 4 cases of hemolytic streptococcus septicaemia. In every instance, during the stage of active infection, agglutination of hemolytic streptococci was demonstrable in dilutions of serum ranging from 1 to 500 up to 1 to 10,000. The capacity to react was not related to the type of infection but seemed to be dependent upon the severity of the disease.

Sera obtained from patients suffering from many chronic diseases have also been investigated. A complete report of the results will be published subsequently. At the present time, it is sufficient to state that many of these tests have been negative and the results, therefore, serve as controls. Additional control observations have been made

TABLE I

Agglutination of hemolytic streptococci by sera from cases of acute bacterial infection

PATIENT	DISEASE	DAY OF DISEASE	TEMPERATURE	LABORATORY AND CLINICAL DATA	DILUTION OF SERUM							
					1:10	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000
Gi	Pneumonia	7th	104	Type III pneumococcus. Septicaemia. Died 8th day	++++	++++	++++	++++	+++	+++	++	±
Ch	Pneumonia	2nd	102	Type II pneumococcus. No blood infection. Recovered	++++	++++	++++	+++	++	±	−	−
Bu	Pneumococcus septicaemia	2nd	106	Type II pneumococcus. Septicaemia following acute otitis media. Died	++++	++++	++++	++++	++++	+++	++	++
Qu	Hemolytic streptococcus septicaemia	10th	105	Onset with acute tonsillitis. Died 2 days later	++++	++++	++++	++++	++++	+++	++	+
Ho	Bacterial endocarditis	?	102	Streptococcus viridans blood infection. Died 6 weeks later	++	+++	++++	++++	+++	+++	+	±
Cr	Typhoid fever	19th	104	Typhoid bacilli recovered from blood, urine and stools. Widal positive, 1:160. Recovered	±	++	++++	++++	++++	++++	+++	++
Do	Meningococcus meningitis	5th	102	Meningococci recovered from spinal fluid. Comatose 8 days. Recovered	++++	++++	++++	+++	+++	++	±	−
Wi	Gonococcus urethritis and arthritis	4 wks.	100	Gonococci seen in urethral pus and recovered from joint fluid. Convalescent	++++	++++	++++	+++	++	±	−	−

Ga	Pulmonary tuberculosis	?	103	Pulmonary hemorrhage, tubercle bac. in sputum in large quantities. Convalescent	++++	++++	++++	++	±	−	−	−
Sw	Undulant fever	6 wks.	100	Intermittent fever. Serum agglutination *Br. melitensis* 1:160. Blood and stool culture negative	++++	++++	+++	++	+	−	−	−
Lu	Lung abscess	6 mos. ?	102	Productive cough 6 months, *Staph. aureus*. No hemolytic streptococci	+	++	++++	++++	++++	+++	+	±
30 normal adults					−	−	−	−	−	−	−	−

Tests were carried out with living organisms. Tubes were incubated in water-bath at 37.5° C. for 2 hours and placed in ice-box overnight.

with sera from thirty normal adults. In every instance there has been a complete absence of reactivity. When patients' sera were being tested, comparable tests were made with normal serum, the same culture being used with dilutions of each serum.

In table I it may also be noted that sera possessing high titres of agglutination were derived from patients during the active febrile stage of the disease. It has been found that the capacity of a patient's serum to react appears soon after the beginning of infection. In a few cases it was possible to obtain serum within 24 to 48 hours after onset (Cases Ch. and Bu. in table I) and in these instances strongly positive reactions were demonstrable. In one case of typhoid fever, the appearance of "agglutinins" for hemolytic streptococci antedated by seven days the development of a positive Widal reaction. These results, therefore, indicate that the alterations of the serum responsible for the reaction appear very soon after initiation of infection.

In many instances, patients were followed by serological tests both during the progress of the illness and also after recovery. In table II are given the results of repeated tests made with serum derived at frequent intervals from a case of typhoid fever and from a case of pneumococcus pneumonia.

From the table it may be seen that the reactivity of the serum paralleled the activity of the infection and that, following recovery, the phenomenon was no longer demonstrable. This same relationship has been observed in all the cases followed through the course of the illness and into the time of recovery. It seems evident, therefore, that the alterations in the serum which cause the streptococci to be clumped is a transient reaction of infection and that recovery, characterized by the restoration of other body processes to normal, is also followed by the disappearance of certain properties of the blood serum. The abnormal state of the blood during infection is also manifested by the suspension stability of the red blood cells (5). The sedimentation rate of red cells is reported as being increased during acute bacterial disease and returns to normal soon after recovery. The parallelism between the occurrence of agglutination of hemolytic streptococci and the sedimentation rate of the red blood cells will be described farther on in this report.

The conditions under which the clumping of streptococci by reactive

TABLE II

Reactivity of serum during course of acute infection

SERUM	DAY OF DISEASE	TEMPERATURE	LABORATORY AND CLINICAL DATA	DILUTION OF SERUM							
				1:10	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000
Case of typhoid fever	19th	104	Typhoid bacilli in blood. Widal positive 1:180. Acutely ill	+	++	++++	++++	++++	++++	+++	++
	24th	104	Condition unchanged	+	++	++++	++++	++++	++++	+++	++
	32nd	101	Improving; temperature swings to normal. Blood culture sterile	+	+++	++++	++++	++++	+++	++	±
	38th	98.6	Temperature normal 4 days	+	++	++++	++++	++++	++	±	−
	45th	101.5	Increased fever, pyelitis. *B. typhosus* occasionally in urine	+	+++	++++	+++	++	±	−	−
	58th	99	Afebrile for 7 days	±	++	+++	±	−	−	−	−
	70th	99	Afebrile for 19 days. Recovered	±	±	+	−	−	−	−	−
Case of pneumococcus (type II) pneumonia	2nd	102	Typical lobar pneumonia. Pneumococcus Type II. Blood culture sterile	++++	++++	++++	++++	+++	++	±	−
	6th	103 to 99	Day of crisis	++++	++++	++++	++++	+++	+	−	−
	10th	99	4 days after crisis	++	++	++	−	−	−	−	−
	14th	99	8 days after crisis	±	±	±	−	−	−	−	−

Tests were carried out with living organisms. Tubes were incubated in water-bath at 37.5°C. for 2 hours and placed in ice-box overnight.

sera is demonstrable depend upon certain characteristics of the organisms and certain properties of the serum. Although investigation of the various factors controlling the phenomenon is as yet incomplete, a limited number of facts have been sufficiently well established to justify presentation.

Strains of hemolytic streptococcus employed. At the present time, four different strains of hemolytic streptococci have been found to be agglutinable in the sera of patients. One of these strains,[2] originally derived from a case of scarlet fever, has been employed in most of the tests. This strain was reactive when first tested. The other three strains were not agglutinable when first isolated, but became so after from 6 to 25 subcultures in plain meat infusion broth. One of these strains was derived from a case of acute follicular tonsillitis and the other two from cases of hemolytic streptococcus septicaemia. Six other strains of diverse origins have been subcultured and tested at repeated intervals but have remained inagglutinable. Differences in biological properties of agglutinable and inagglutinable cultures are being investigated.

Culture media employed. Early in the investigation it became evident that the media used for cultivation influence the development of agglutinable cultures. The usual meat infusion broth, adjusted to pH 7.6, and containing 0.02 per cent to 0.05 per cent dextrose has been found the most satisfactory. The final pH, after growth has occurred, has never gone below 7.2. When the concentration of dextrose is made 0.2 per cent or more, the pH of full-grown cultures usually drops below 6.0. These cultures are not reactive. Some evidence is available which indicates that the degree of acidity of the culture medium affects the reactive constituent of the serum and not the bacteria. This effect of the pH is suggested by the following experiment: Agglutinable organisms were removed from the culture medium by centrifugation and resuspended in sterile broth of pH 6.0. After standing 4 to 5 minutes the resuspension was tested with reactive sera and found to be inagglutinable. However, when the inagglutinable organisms in the medium of pH 6.0 are centrifuged out and again resuspended in broth of pH 7.6, agglutinability is restored.

[2] This strain, sent to us through the courtesy of Dr. M. H. Dawson, College of Physicians and Surgeons, Columbia University, is a subculture of the scarlatinal streptococcus isolated by Dr. A. R. Dochez and known as N. Y. 5.

Although the pH of the medium in which the organisms are suspended influences the reaction, other as yet inexplicable factors may inhibit agglutination. This is evidenced by the fact that in some lots of broth, although adjusted to the proper pH, the streptococci are not agglutinable. It has been suggested that reactive cultures are those which tend to grow slightly granular and that serum exaggerates this "natural" condition. Examination of cultures with a magnifying lens sometimes shows fine granulation. However, cultures of other strains which are indistinguishable in appearance from the test strains,

TABLE III

Effect of heat on agglutinability of hemolytic streptococci*

TEMPERATURE	DURATION OF HEATING	DILUTIONS OF SERUM					VIABILITY ON BLOOD AGAR
		1:10	1:50	1:100	1:500	1:1,000	
°C.							
	—	++++	++++	++++	++++	+++	Innumerable colonies
56	1 hour	—	—	—	—	—	No growth
56	5 minutes	++++	++++	++++	++++	+++	Innumerable colonies
56	10 minutes	++++	++++	++++	++++	+++	Innumerable colonies
56	15 minutes	—	—	—	—	—	No growth
56	20 minutes	—	—	—	—	—	No growth
45	1 hour	++++	++++	++++	++++	+++	Innumerable colonies
50	1 hour	++++	++++	++++	++++	+++	Innumerable colonies
52	1 hour	++++	++++	++++	++++	+++	Innumerable colonies

* Test serum derived from case of pneumococcus (Type III) pneumonia.

Agglutination tests incubated in water-bath at 37.5°C. for 2 hours and placed in ice-box overnight.

and even a more granular growth, are not influenced by reactive sera.

Effect of heat on agglutinability of streptococci. The serological reactions were always carried out with living organisms and were incubated in the water-bath at 37.5°C. In order to determine the effect of heat on agglutinable cultures, reactive strains were subjected to various temperatures and retested. The results are recorded in table III.

From the table it may be seen that heating the organisms at 56°C. long enough to cause death destroys agglutinability. On the other

hand, heating cultures at 52°C. or less for an hour neither killed the bacteria nor altered reactivity. By heating small amounts of culture at 56°C. and testing at 5-minute intervals, it has been possible to determine with some accuracy the point at which agglutinability is lost. On repeated investigation the length of heating necessary to destroy reactivity of cultures has been found to coincide with the thermal death-point. In this respect the reaction differs from other serological tests with streptococci. A review of the literature reveals the fact that heat-killed bacteria were frequently employed in testing for the presence of antibodies in anti-sera made by the immunization of animals. The effect of heat on the test cultures is, therefore, one of the unique characters of the reaction.

Effect of formalin on the agglutinability of streptococci. In view of the effect on the reaction of heat-killing the cultures, observations were also made with organisms killed with formalin. The addition to cultures of 40 per cent stock formalin in a quantity necessary to make a final concentration of 0.3 per cent proved to be bactericidal for the test strains. The following experiment, the results of which are recorded in table IV, was then carried out. A culture of agglutinable streptococci was divided into 3 parts. One part remained untreated, the second part was heated at 56°C. for 1 hour; to the third part sufficient formalin was added to make a 0.3 per cent solution. Each part was then tested with reactive sera and also subcultured to determine viability.

The results show that the agglutinability of cultures killed with formalin is not affected, although heat-killed streptococci are rendered inert. This observation was further amplified by testing organisms which had first been killed with formalin and then heated at 56°C. for 1 hour. Under these conditions it was found that heating destroys the agglutinability of formalinized as well as of living cultures.

PROPERTIES OF REACTIVE SERA

From the results so far presented it is evident that the agglutination of hemolytic streptococci by sera from cases of acute bacterial disease differs in certain respects from the usual immunological reactions. In attempting to analyze the mechanism further, interest has been directed to the properties of reactive sera. This study is not yet complete, but such facts as have been established are presented and they

lend additional support to the view that the phenomenon does not involve a "true" anti-streptococcal antibody.

Absorption. Live organisms contained in 400 cc. of a full-grown broth culture were removed by centrifugation and resuspended in a 1 to 10 dilution of reactive serum. After the tubes had been incubated in the water-bath at 37°C. for 2 hours and allowed to stand in the ice-box overnight, the agglutinated bacteria were separated by centrifugation. The 1 to 10 dilution of absorbed serum was then retested with agglutinable cultures. By this procedure it was found that the "agglutinins" could be completely removed. However, when agglutinable cultures that had been rendered inert by heat were used for absorption, the reactivity of serum was not affected.

Sera from patients with typhoid fever usually agglutinated both hemolytic streptococci and typhoid bacilli. Sera possessing these properties have been absorbed with each of the reactive strains. It was found that removal of one antibody did not affect the reactivity of the serum against the heterologous culture.

The results of the experiments with absorption demonstrate that the constituent of serum on which reactivity depends may be specifically absorbed.

Serum constituent containing reactive factor.[3] In attempting to demonstrate additional differences between the clumping of streptococci and true antibody reactions, a method of fractionating serum proteins was employed. This experiment is part of an investigation of the similarity between the streptococcal phenomenon and so-called conglutination, and was stimulated by the fact that conglutination differs, in certain respects, from specific antigen-antibody reactions. The term conglutination was originally applied by Bordet and Streng (1) to a phenomenon exhibited by beef serum. They found that when beef serum, which alone produced no hemolysis or agglutination, was mixed with red blood cells plus specific antibody plus complement, antibody action was both accelerated and augmented. This result has been confirmed and extended by others who have found conglutinin present in different animal sera and noted that bacterial agglutination

[3] The authors wish to express their appreciation to Dr. Harry Eagle of the Department of Syphilis for his valuable suggestions and technical assistance in carrying out the following experiments.

TABLE IV

Effect of formalin and heat on agglutinability of hemolytic streptococci

SERUM		LIVE CULTURES				HEATED 56°, 1 hour				FORMALINIZED (0.3 PER CENT)			
Patient	Disease	1:10	1:100	1:500	1:1,000	1:10	1:100	1:500	1:1,000	1:10	1:100	1:500	1:1,000
Ho	Pneumococcus pneumonia	++++	++++	++++	++++	−	−	−	−	++++	++++	++++	++++
Ot	Typhoid fever	++	++++	++++	++++	−	−	−	−	++	++++	++++	++++
Ri	Typhoid fever	+++	++++	++++	++++	−	−	−	−	+++	++++	++++	++++
Ma	Hemolytic streptococcus septicaemia	++++	++++	++++	++++	−	−	−	−	++++	++++	++++	++++
Tested for viability on blood agar		Innumerable colonies				No colonies				No colonies			

All tests incubated in water-bath at 37.5°C. for 2 hours and placed in ice-box overnight.

TABLE V

Separation of serum constituents responsible for agglutination of hemolytic streptococci and typhoid bacilli*

	HEMOLYTIC STREPTOCOCCUS							TYPHOID BACILLUS					
	Dilutions of serum												
	1:10	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:10	1:20	1:40	1:80	1:160	1:320
Whole serum	+	+++	++++	++++	++++	+++	+	+++	+++	+++	++	+	−
CO_2 precipitable fraction	+	+++	++++	++++	++++	++	±	+	±	−	−	−	−
Non-precipitable fraction	++	±	−	−	−	−	−	+++	+++	++	+	±	−

* Serum derived from case of typhoid fever.

Living cultures of streptococci, formalinized cultures of typhoid bacilli employed.

All tests incubated in water-bath at 37.5°C. for 2 hours and placed in ice-box overnight.

may be influenced as well as hemoagglutination. Experiments with reactive patients' sera have, therefore, been done which were based on investigations of conglutination. The results presented, which concern this part of the problem, are limited to the chemical fractionation of serum.

In the experiments to be described, serum fractionation was carried out according to the method described by Liefmann (2) and employed by Dean (3), Eagle (4), and others in investigating conglutination. To 1 cc. of serum, kept in the ice-box, 9 cc. of cold distilled water are added. CO_2 is then allowed to bubble through the mixture. A white cloud immediately appears which increases in intensity for 3 to 5 minutes. The maintenance of low temperature induces the formation of a granular precipitate. The precipitate, removed by centrifugation, is redissolved in 10 cc. of physiological salt solution. The supernatant fluid, after being adjusted to pH of 7.6, is restored to 0.85 per cent salt concentration by the addition of 1/19 its volume of 17 per cent NaCl solution. The two fractions, as well as the original serum, were then titred against suitable cultures of streptococci. Sera from 7 different patients have been fractionated and tested in this manner. In every instance, the portion of the euglobulin fraction precipitated by CO_2 was found to be almost as reactive as the whole serum, whereas the unprecipitated portion gave only weak agglutination in the strongest concentration. It seems probable that, if the process of precipitation had been carried on longer or had been repeated, a complete separation of the anti-streptococcal constituent could have been effected. The sera tested were derived from 3 patients with typhoid fever, 2 with pneumococcus pneumonia, 1 with gonococcal urethritis and arthritis, and 1 with hemolytic streptococcus septicaemia. Table V records the results with serum obtained from a case of typhoid fever. Tests with this serum were particularly instructive, since agglutination of both typhoid bacilli and hemolytic streptococci was demonstrable.

The results presented in the table demonstrate that the serum constituent on which streptococcus agglutination depends may be separated from the portion responsible for agglutination of typhoid bacilli. Since the Widal test has other characteristics of a true antibody-antigen reaction, the clumping of streptococci by the same serum is referable to different properties of the serum.

In the chemical separation of the serum proteins, the antistreptococcal constituent was obtained by the method used by others for isolating conglutinin and also complement mid-piece. Eagle (4) has shown that conglutination is a property of the fraction of complement which has been termed mid-piece. The implication, therefore, that the phenomena exhibited by this serum fraction are closely related, is obvious, and additional observations on the correlation are now in progress. However, a consideration of the components used in eliciting each reaction demonstrates at least one distinct difference. In reactions designed to demonstrate conglutination specific anti-sera are augmented by the addition of a second serum (conglutinin) whereas patients' sera agglutinate streptococci without the addition of other material. The interpretation of the streptococcal agglutination as an example of conglutination therefore, requires the presence in reactive patients' sera of a true antibody which is augmented by conglutinin also present in the same sera.

COMPARISON OF STREPTOCOCCAL AGGLUTINATION WITH SUSPENSION STABILITY OF THE BLOOD

The increased sedimentation rate of red blood cells is a phenomenon which occurs in many diseases of diverse etiology. In acute bacterial infections the suspension stability of the blood has been found to be altered during the active phase of disease and to return to normal after the patient's recovery (5). Since the capacity of patients'sera to agglutinate streptococci appears and disappears under conditions comparable to those which influence the rate of red-cell sedimentation, the two tests have been performed concurrently. In all of the cases of acute infection followed in this manner, a striking parallelism was found to exist between sedimentation rate of red cells and capacity of serum to clump streptococci. Attempts to associate directly the two reactions with a common property of the blood have been made by absorption tests. Plasma from a sample of blood exhibiting increased sedimentation rate was mixed with the agglutinable streptococci contained in 500 cc. of culture and placed in the water-bath at 37.5°C. for 1 to 2 hours. The organisms were then removed by centrifugation and the plasma restored to the red cells. After careful mixing, the sedimentation of the red cells in the absorbed plasma was determined.

Other samples of plasma from the same blood were similarly absorbed with non-agglutinable pneumococci, remixed with red cells, and the suspension stability retested. Up to the present time, experiments of this character have been done with blood from 7 patients. In 3 instances, the sedimentation rate after absorption with streptococci has been completely restored to normal; in two instances the rate was only partially affected and in 2 others no change in the velocity of sedimentation occurred. In none of the experiments did absorption with pneumococci alter the suspension stability of the blood. Difficulties in technical procedure have in many of the tests made the results uncertain and a final interpretation must await the development of a more exact technique. In spite of this, the results have been sufficiently suggestive to justify a continuation of this part of the investigation.

DISCUSSION

The observations recorded in this communication demonstrate that the sera of patients acutely ill with bacterial infections are capable of agglutinating certain strains of hemolytic streptococci. A mixture of suitable streptococci with reactive sera results in the formation of coarse clumps of bacteria, which, in gross appearance, cannot be differentiated from true agglutination. In spite of this, the phenomenon differs, in many respects, from specific antigen-antibody reactions and the unusual features are evidenced not only by certain characteristics of agglutinable organisms but also by certain properties of reactive sera.

The patients whose sera agglutinated streptococci were suffering from acute diseases of diverse bacterial origin. The group of cases comprising this investigation was limited to diseases in which a bacteriological diagnosis was definitely established. The sera of patients having pneumococcus pneumonia or typhoid fever, or other bacterial infections, were reactive to the same degree as serum from cases of hemolytic streptococcus septicaemia. The capacity of a patient's serum to react was not related to the type of bacterial incitant of disease and, from the etiological standpoint, was entirely nonspecific. In the group of cases studied, the severity of the illness rather than the genus of causative bacteria determined the degree of serum reactivity.

When repeated tests were made during the course of an acute infection, the ability of serum to agglutinate streptococci was found to appear early after onset, to persist during the active phase of disease, and to disappear very soon after recovery. This course of events differs strikingly from the well-known immune response exhibited by patients who, as a result of infection, develop, late in the disease, antibodies specific for the etiological agent.

In attempting to analyze the mechanism underlying the phenomenon, interest has been directed to both organisms and sera. The strains of agglutinable streptococci have been derived from four different sources and seem to possess no disease specificity. A study of the biological properties of agglutinable and non-agglutinable strains is now in progress and will be subsequently reported. At the present time it is interesting to note that, when agglutinable cultures are heated to the thermal death point, agglutinability is lost; however, when similar cultures are killed with formalin, the reactivity is unaltered.

The cultural conditions which best promote the development of agglutinable streptococci is not yet clear. Even with the most favorable medium, the cultures may vary in agglutinability with different "lots" of broth. Although no direct evidence is available it seems probable that the agglutinability of cultures depends on some physical property of the streptococci rather than on the presence of a reactive heat-labile organic constituent of the cells.

That the streptococcal agglutination described involves immunological principles different from specific anti-bacterial reactions receives additional support from the results of chemical fractionation of reactive sera. For example, a similarity between the streptococcal reaction and so-called conglutination is evidenced by the fact that the part of the euglobulin fraction of serum which is precipitated, under proper conditions, by CO_2 and which contains conglutinin (4), also contains the constituent responsible for clumping streptococci. In chemical fractionation experiments, sera from patients with typhoid fever have been particularly interesting. In these cases the whole serum agglutinated both hemolytic streptococci and typhoid bacilli. However, the CO_2 precipitable fraction of the same serum contained the streptococcal reacting constituent and the supernatant fluid

retained the fraction reactive with typhoid bacilli. Since the typhoid bacillus agglutination possesses properties of true antigen-antibody reaction, the clumping of streptococci by the same serum requires a different interpretation.

The occurrence of the phenomenon under consideration has been of interest not only because of contrasts to other immunological reactions, but also because of a similarity to certain properties of the blood manifested by other tests. Alterations in the physical properties of blood during acute infections have been recognized by testing the suspension stability of the red blood cells. The occurrence of an increased sedimentation rate, as described by others and also observed in this investigation, parallels in acute bacterial disease the streptococcal agglutination. That the two phenomena might rest upon a common property of blood constituents therefore suggested itself, and attempts to determine this point by absorption tests have yielded suggestive results. Reimann (6) has recently reported that plasma from patients whose blood showed an increased sedimentation rate induced a microscopical clumping of several different strains of bacteria. He attributed the effect of the plasma on the bacteria to the changed viscosity of the blood which presides over the suspension stability of the red cells.

Although final analysis is incomplete, the results so far obtained indicate that the mechanism underlying the reaction depends upon characteristics of both serum and bacteria. With respect to serum, reactivity is interpreted as an alteration of the blood induced by and paralleling the active disease process. With respect to the bacteria, reactivity is interpreted as dependent on the fact that agglutinable streptococci are particles possessing physical properties which render them susceptible to the altered blood serum. If this hypothesis proves correct, it should be possible to find other suitable particulate matter, both animate and inanimate, capable of being similarly affected by sera from patients with acute infection.

CONCLUSIONS

1. Sera from patients acutely ill with a variety of bacterial infections are capable of agglutinating certain strains of hemolytic streptococci.
2. During the course of an acute illness, the capacity of a patient's

serum to react is demonstrable early after onset, persists during the active stage, and disappears soon after recovery.

3. Four strains of *Streptococcus hemolyticus*, derived from different diseases, are agglutinable.

4. Agglutinability is destroyed by heating cultures to the thermal death point. Agglutinable cultures killed with formalin are not altered.

5. The protein fraction of serum precipitated by CO_2, under the conditions described, contains the constituent reactive with streptococci. By this method, sera from cases of typhoid fever were fractionated and the agglutinins for typhoid bacilli and hemolytic streptococci were separated.

6. The implications suggested by the parallelism of altered suspension stability of red blood cells and streptococcal agglutination are discussed.

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After this article had been submitted for publication, the authors' attention was called to a report on Agglutination of Streptococci by G. H. Weaver, J. Inf. Diseases, 1904, 1, 91. Weaver found that sera from patients with pneumonia and typhoid fever agglutinated scarlatinal strains of hemolytic streptococci equally as well as did sera from cases of scarlet fever. He also noted that heating the organisms 60 to 62°C. destroyed agglutinability.

DIRECT MEASUREMENTS OF THE OXYGEN CONSUMPTION OF ISOLATED, BEATING AURICLES FROM NORMAL AND THYROTOXIC GUINEA-PIGS

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The mechanism and the site of action of thyroxine have not been established although there is growing evidence that in the intact organism the substance affects directly the metabolic processes of the living cell. Lewis and McEachern (24) showed that the hearts of thyrotoxic animals continued to beat, even when isolated, at a greatly enhanced rate. This seemed to indicate the persistence of the specific thyroid effect upon the isolated tissue. Andrus and McEachern (2) found that isolated beating auricles from thyrotoxic animals were much more sensitive to deprivation of oxygen and to exposure to isotonic solutions of sodium lactate than were similar preparations from normal animals. These results suggested that some alteration had occurred in the metabolic processes of the auricles from thyrotoxic animals. The present report deals with measurements of the oxygen consumption of isolated beating auricles from normal and thyrotoxic guinea-pigs.

METHOD

Healthy male guinea-pigs were used; temperature, weight and heart rate were recorded every 2nd or 3rd day. All animals were kept under similar environmental conditions. Hyperthyroidism was induced by 'Thyroxin' (Roche) which was administered intramuscularly at intervals of 1 to 3 days and in doses of .05 mg. to 0.4 mg., the total dosages varying from .15 mg. over a period of 11 days to 3.275 mg. over 45 days. In the thyroxine-treated animals there was a rapid fall in weight, an increase in heart rate and sometimes an elevation of temperature. Each animal was killed by a blow on the head; the heart was then quickly removed (within 35 to 50 seconds)

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and placed in iced Ringer-Locke's solution (pH 7.4). The auricles were carefully detached and placed in the bulb of the micro-respiration apparatus which contained 3 c.c. of Ringer-Locke's solution (pH 7.4) at 37°C. Spontaneous rhythm usually commenced at once.

The micro-respiration apparatus used was similar to that described by Winterstein (30) but slightly modified by Harrop and Barron (16). The vessels were of 12 c.c. capacity and were fitted with side chambers containing 0.5 c.c. of 6 per cent KOH into which filter-paper wicks were inserted. The tissue was submerged in 3 c.c. of Ringer-Locke's solution in one vessel and an equalizing volume of Ringer-Locke's solution was placed in the equilibrating vessel. The Ringer-Locke's solution was adjusted to pH 7.4 with either phosphate or bicarbonate buffer. The former was used most frequently and was most satisfactory in keeping the pH of the solution constant. There appeared, however, to be no significant difference in oxygen consumption when one or the other was used. The entire apparatus was submerged in a constant temperature water-bath at 37°C. $\pm 0.1°$ and was shaken at a rate of 80 per minute. Pure oxygen was introduced into the vessels and 20 to 30 minutes allowed for equilibration. Readings were then commenced and were made at 20 or 30 minute intervals. The present figures are based, in each instance, upon the average of the readings taken during the first two hours. During this period the oxygen consumption was usually maintained at a satisfactorily even level. After each experiment the auricles were blotted upon filter paper and weighed. They were then dried over night at 60°C. and reweighed. The ratio of wet weight to dry weight was variable but averaged about 6:1. Dry weights have been used entirely in this report for computing the rates of oxygen consumption.

It is of interest that the average ratio of wet weight to dry weight was 5.8 for the thyroxinized series and 6.4 for the normal series. This would indicate that the increase in heart weight noted in hyperthyroid animals by many observers (5, 17, 18, 19, 20, 21, 28) is due to actual hypertrophy and not merely to an increased water content.

RESULTS

The auricles from thyrotoxic animals were found to consume more oxygen per gram of tissue than auricles of similar weight from normal

animals. Table 1 summarizes the results obtained in 31 experiments on normal auricles and in 15 experiments on auricles from thyrotoxic animals.

It is apparent that the larger auricles used less oxygen per gram of tissue than the smaller ones. There are two possible explanations for this: (1) that the larger, and therefore thicker, auricles do not allow complete diffusion of oxygen into the tissue. This explanation suggests itself in view of the fact that the thickness of the auricular wall in the guinea-pig occasionally exceeds that demanded by the Warburg formula. It must be pointed out, however, that much thicker auricles (rabbit's, cat's, dog's) continue to beat for hours in an oxygenated bath of Ringer-Locke's solution without evidence

TABLE 1

Average oxygen consumption of auricles from normal and thyrotoxic guinea-pigs in c.c. per gram (dry weight) per hour

	WEIGHT OF AURICLES (DRY) IN MGM.		
	10–20	21–30	31–40
	O_2 use in c.c. per gm. per hr.	O_2 use in c.c. per gm. per hr.	O_2 use in c.c. per gm. per hr.
Normal	4.75	3.96	3.18
Thyrotoxic	5.07	4.40	3.84
Per cent increase of O_2 use in thyrotoxic series	7.3%	11.1%	20.7%

of anoxemia. (2) That the larger auricles come from older animals and for this reason have a lower rate of oxygen consumption. Gerard (14) found this to be the explanation of similar variations in oxygen consumption which he observed in nerves of different sizes.

If the first explanation is correct, the effect would be to make the difference of oxygen consumption between the two series (normal and hyperthyroid) appear less than is actually the case.

Inasmuch as isolated auricles from thyrotoxic animals beat at a faster rate than similar preparations from normal animals, it is of interest to know whether the increased oxygen consumption of the former preparations is due solely to the augmented rate or to some inherent alteration in the tissue. From the present experiments it is impossible to answer this question definitely.

In a number of experiments the auricles ceased to beat during the period of observation. No significant change in the rate of oxygen consumption accompanied this. In cold-blooded animals Clark and White (6, 7) have noted that there is very little difference in the oxygen consumption of contracting auricles and of auricles at rest, providing they are empty and not performing work. These facts suggest that in the present experiments the increase in rate may not be alone responsible for the increase in oxygen consumption.

Evidence which corroborates the above results has been obtained recently by Lewis and Dock at Stanford University School of Medicine (23). These workers have measured the oxygen consumption of heart-lung preparations from 20 normal and 20 thyrotoxic rats. One half the experiments in each series were done under chloretone anaesthesia and the other half without an anaesthetic save for a brief period of etherization at the beginning of each experiment. In the chloretone group the total metabolism of the rat was on the average 34 per cent higher in the thyroid-fed series than in the normal controls. In the thyroid-fed series the oxygen consumption of the heart-lung preparations was 36 per cent greater, the heart weight 18 per cent greater and the heart rate 13 per cent greater than the corresponding figures for the normal control series. In the group of experiments done without anaesthesia the results were practically the same. The oxygen consumption per gram per beat was almost identical in the thyroid-fed and in the normal series. Lewis and Dock concluded that the increased rate of oxygen consumption which they noted in preparations from the thyroid-fed group could be accounted for by the increase in heart weight and heart rate. It is possible that the increase in oxygen use may parallel the increase in heart rate. This, however, does not necessarily indicate that the increase in oxygen consumption is due alone to the increase in rate, since both phenomena may have a common origin in some fundamental metabolic change in the tissue.

It would seem possible that this question could be answered by the use of minced or thinly sliced cardiac or skeletal muscle from normal and hyperthyroid animals. The rate of contraction would then be definitely ruled out as a cause for any variations observed in the oxygen consumption. With this end in view, a large number of

experiments were performed upon skeletal and cardiac muscle tissue that had been removed from normal and hyperthyroid rabbits and guinea-pigs. In some instances the tissue was chopped into very small particles and in other instances cut into very thin slices according to the technique described by Warburg (29). The rate of oxygen consumption in these experiments was usually high (5 to 10 cc. per gm. (dry wt.) per hr.) during the first twenty-minute period, but in every instance fell away rapidly and at the end of two hours was frequently but a half or a quarter of the original level. From this point on the oxygen utilization decreased slightly until the end of a five-hour period when the experiment was usually terminated because of the onset of bacterial growth. It was felt that these rapidly falling curves must have been the result of injury to the tissue and it was considered unsafe to draw any conclusions from them. Owing to the great length of muscle cells it is probably impossible to slice the tissue without causing severe damage. It would seem wise, therefore, to adopt a guarded attitude toward the data obtained from the use of such tissues.

DISCUSSION

The site of action of the active agent embodied in thyroxine is not definitely known. The increased rate of metabolism which follows the administration of thyroid products may be explained as due to the action of the substance either directly upon the tissues or upon some intermediate organ which in itself stimulates cellular oxidations.

Aub, Bright and Uridil (4), in 1922, showed that the elevation of metabolism produced by thyroxine could not be explained by muscular activity, muscular fibrillation or increased muscle tonus, and that the adrenal glands were not necessary for the maintenance of the elevated metabolism. Haffner (15) showed that the metabolism of hyperthyroid animals was excessive even after the spinal cord had been cut in the cervical region and the body therefore freed of impulses from the heat-regulating center. Ring, Dworkin and Bacq (26) tested the effect of complete sympathectomy upon the response of animals to injections of thyroxine. By removing the entire thoracolumbar sympathetic chain on each side it was possible to free a large part of the body of impulses from the sympathetic nervous system.

The elevation of metabolism following injections of thyroxine was the same in the sympathectomized and in the normal animals. Finally Riml and Wolff (25) in an extensive series of experiments on dogs have shown that, even after extirpation of the sympathetic and parasympathetic fibres and ganglia, and after section of the nerves to the extremities, the characteristic elevation of metabolism occurs upon administration of thyroid substance. In their experiments ample time was allowed for the nerve fibres to degenerate before thyroid feeding was commenced.

More direct evidence has been obtained by the study of isolated tissues *in vitro*. Such a variety of methods has been used to demonstrate metabolic changes in surviving tissues that the results are difficult to compare. A number of workers have studied the oxygen consumption of living cells by manometric methods and claim to have observed increases in oxygen use following the addition of thyroxine or thyroid substance to the isolated preparation. A large number of observers have failed to confirm this finding. "Acute" experiments of this type have been performed upon plant seedlings, upon amoebae and upon a variety of tissues from normal animals. We are not aware of any convincing evidence that thyroxine can exert its specific effect upon isolated cells *in vitro* within the short periods of observation that obtain in "acute" experiments. Whether an increased rate of metabolism can be demonstrated in a tissue following its prolonged exposure to thyroxine is a question yet to be answered. It is possible that thyroxine may be able to exert its action only through the agency of the intact organism.

More conclusive information has been gained through a comparison of the metabolism of tissues removed from hyperthyroid and normal animals. Rohrer (27) in 1925 measured the oxygen consumption of organs (liver, kidney, muscle) isolated from normal and thyroid-fed white mice and found increases of 15.6 to 23.9 per cent in the oxygen use of the tissues from hyperthyroid animals. These experiments were performed with the intact organs suspended in oxygen in a Krogh micro-respirometer, hence any error resulting from too great thickness of the tissues would tend to make the observed difference between the normal and hyperthyroid series less than the actual difference. Foster (12), using the Warburg method, found that

diaphragm muscle from thyroidectomized rats consumed from 25 per cent to 30 per cent less oxygen than similar tissue from normal rats. His experimental animals were, however, as Dye and Waggener (11) have pointed out, heavier than his normal rats, weighing 155 to 241 grams as compared with 142 to 180 grams for the controls. Dye and Maughan (9) using volumetric methods found that the respiration of skeletal muscle from cretin pups was 25 to 60 per cent less than that of similar tissue from normal animals. Dye and Waggener (11) studied a variety of minced tissues from normal and thyroidectomized lambs and pups to determine their relative power to catalyze the formation of indophenol from a substrate composed of a-naphthol and dimethyl-paraphenylene-diamine-hydrochloride in alkaline solution. A marked diminution in indophenol oxidase was found in all the tissues from thyroidectomized animals. These tissues were also found by Dye and Maughan (10) to have a decreased power of oxidizing succinic acid as compared with similar tissues from normal animals. It should be noted that all of the tissues used in the three groups of experiments last referred to were studied after being finely minced. In view of this fact it is doubtful if they can be regarded as surviving tissues.

In common with many other observers Dresel (8) was unable to demonstrate any increase in oxygen use or glycolysis in isolated tissues to which thyroxine had been added *in vitro*. On the other hand, liver and kidney tissues removed from animals (full-grown rats) five hours after the subcutaneous injection of thyroxine showed an increased oxygen consumption. For liver this increase amounted to 60 per cent when the tissue was removed 24 hours after the injection of thyroxine, 100 per cent at 48 hours and 200 per cent at 72 hours. It is noteworthy that Anselmino, Eichler and Schlossmann (3), in a careful series of experiments, found little or no increase in the oxygen consumption of liver, kidney and spleen tissues removed from white rats 1 to 7 days after the subcutaneous injection of 1.0 mg. of thyroxine. Their experiments were done by the Warburg method.

In general the evidence would seem to point to an increase in the metabolic activity and the oxygen consumption of the tissue cells of animals which have been rendered hyperthyroid with thyroid products. It would also appear that these changes persist and are

measurable in surviving tissues following their isolation from the animal body. Without entering into a discussion of the subject it is of the greatest interest to note that many of the workers (1, 13, 15, 22) who accept this view suggest that the increased oxygen consumption is secondary to a more important and fundamental phenomenon—an increase in the rate of anaërobic glycolysis in the tissue cells.

SUMMARY

Direct measurements were made of the oxygen consumption of isolated beating auricles from normal and thyrotoxic guinea-pigs by means of a modified Barcroft-Warburg micro-respirometer. In 31 experiments on normal auricles and in 15 experiments on auricles from thyrotoxic animals the latter preparations were found to have a higher rate of oxygen consumption than preparations of similar weight from normal animals. For auricles weighing (dry weight) 10–20 mg. the increase averaged 7.3 per cent, for auricles weighing 21–30 mg. 11.1 per cent and for auricles weighing 31–40 mg. 20.7 per cent. The average of the maximum rates of contraction was 267 per minute for the normal series and 304 per minute for the thyrotoxic series. A brief review is given of the literature relating to the site of action of thyroxine in the animal organism.

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BOOK REVIEWS

Grow Thin On Good Food. By LUELLA E. AXTELL, M.D. 1930. (*Funk & Wagnalls Co., New York.*)

Dr. Axtell's book is intended as a *vade mecum* for those anxious to reduce established or threatened over-weight. The *via crucis*, like most works of this kind, is by the route of proper understanding of food values, carefully planned menus and rational exercise. The tone of the book is purposely bantering—to arrest attention no doubt—with playful illustrations, and the usual hope-inspiring tables to measure or check progress.

W. G. S.

You—and the Doctor. By JOHN B. HAWES, 2nd, M.D. 12°. 181 pages. $2.00. (*Houghton Mifflin Co., Boston, Mass.*, 1929.)

The author's purpose is to interest readers "to take part in the management of their health and not leave it all to the doctor." Rather a readable book, and one that should prove helpful to both patient and doctor.

W. G. S.

Living the Liver Diet. By ELMER A. MINER, M.D. With an Introduction by William P. Murphy, M.D., Instructor in Medicine at the Harvard Medical School, Boston, Mass. 106 pp., $1.50. (*C. V. Mosby Co., St. Louis, Mo.*, 1931.)

This slender volume sets forth the merits of the Minot-Murphy Liver Diet in conditions of pernicious anemia. Himself a pernicious anemia patient, the author details the benefits derived from this form of diet in his own case, and comes to the conclusion that the liver diet he advocates means life for the patient, and "should be part of the daily diet throughout life."

Regularity in the diet is of prime importance. Repugnance, if existing, must be overcome to insure lasting results. According to the author, gratifying results from the liver diet have also been observed in pellagra, achlorhydria, colitis, sprue, beriberi and other diseases.

W. G. S.

The Young Doctor Thinks Out Loud. By JULIAN P. PRICE, M.D. 19½°. 187 pp. (*D. Appleton & Co., New York*, 1931.)

Chiefly of interest to those desirous of taking up a medical career. Premedical education, medical school curricula, internship, and the chances and opportunities of the young doctor in general or in specialized practice are frankly discussed and evaluated, with a final chapter on medical idealism.

W. G. S.

Aspects of Age and Disease. By SIR HUMPHREY ROLLESTON. 304 pp. 8°. (*Macmillan, New York, 1929.*)

To anyone who is familiar with the serious medical studies of Rolleston, this volume offers an opportunity for a more intimate chatty companionship with the author. Here are pleasant, genial discourses on men and nature, revealing the less formal, but therefore more significant, intuitive reactions of the author's personality. To Americans particularly, the book is a timely reminder of the uses of culture.

S. W.

Modern Aspects of the Diagnosis, Classification and Treatment of Tuberculosis. By JAY ARTHUR MYERS. With an Introduction by David A. Stewart. 271 pp. 23½°. $3.75. (*Williams & Wilkins Co., Balto., 1927.*)

This, for students and for those who have not followed the pertinent literature, is an excellent introduction to the subject. The writer's style immediately engages the interest of the reader, making the study of the volume a pleasure rather than a task.

S. W.

The Diet Book. For Doctor, Patient and Housewife. With Specimen Menus for One Week and Recipes. By MARGUERITE REQUA REA (MRS. ALEC. L. REA), with a foreword by Sir James Purves-Stewart, K.C.M.G., M.D. (Ed.), F.R.C.P. (London). 197 pp. $2.75. (*Oxford Univ. Press. London, 1931.*)

Another contribution to the long list of dietaries recommended for illness and convalescence. The author draws largely upon her own experience in sick-room routine, based on studies on the dietetics of Drs. J. Friedenwald, Ruhräh, and others. A well-arranged index of recipes is appended.

W. G. S.

Modern Otology. By JOSEPH CLARENCE KEELER, M.D., F.A.C.S. 858 pp. $10.00. (*F. A. Davis Co., Phila., 1930.*)

The practitioner, as well as the student, will find this a very useful and helpful book. It is arranged in twelve parts, each followed by a bibliography, which pretty well covers the best of the modern literature on the ear and its diseases. The section devoted to otology in children is especially to be commended. In the terminal section, medico-legal aspects of otology are discussed.

Some statements in the text are open to criticism. In discussing calcareous deposits in the tympanic membrane, the author suggests quite properly that there may also be similar deposits in the vicinity of the foot-plate of the stapes, but the reader is led to infer that the latter condition is synonymous with oto-sclerosis. The book is conveniently arranged and well indexed. There are some twenty-five typographical errors.

One might wish that more stress has been laid on lip-reading in the discussion of the physician's duty to his deaf patient.

J. J. C.

A Manual of Normal Physical Signs. By W. P. BLANTON, B.A., M.D. 2nd edition. 237 pages. 12°. $3.00. 1930. (*C. V. Mosby Co., St. Louis, Mo.*)

This little textbook should prove of tremendous help to the medical student. It gives a brief compilation of the normal physical signs without coupling them with pathologic signs. It is written in notebook form, makes an extremely good skeletal arrangement, and should be of great aid in helping the average beginner to find himself in the subject.

P. P. S.

Burns. Types, Pathology, and Management. By GEORGE T. PACK, B.S., M.D. Fellow of the Memorial Hospital, New York City, and A. HOBSON DAVIS, B.S., M.D. Instructor in Pathology, Univ. of Alabama. 60 illust. 364 pages. (*J. P. Lippincott Co., Phila., Pa.*)

The subject matter comprises: (1) fundamental facts concerning burns and scalds, (2) the management of thermal burns, and (3) regional burns; burns by specific agents. The first part is an outline of the history, incidence, burning agents, and general classification of burns. The material is not new, but is assembled in a readily available form. As a compilation covering all practicable methods of treating burns, the second and third parts of the book will prove useful to those called upon to treat such conditions. Recognized procedures are described in detail with their advantages.

It is unfortunate that this compilation came from the press just before the publication of the recent important studies on the physiological chemical changes produced by burns. One feels a lack of critical discussion and physiological background which would have made the volume not only an index, but also a stimulus toward further investigation.

E. M. B.

Physical Diagnosis. By WARREN P. ELMER, B.S., M.D., and W. D. ROSE, M.D. Sixth edition. 903 pages. 337 illustrations. $10.00. (*C. V. Mosby Co., St. Louis, Mo.*)

The volume deals with the technic of physical examination and of normal and abnormal physical diagnosis. It is conveniently arranged in two parts; part one dealing with the technic of physical examination and normal physical diagnosis; part two, with the physical diagnosis of disease.

The text is very well outlined with fluoroscopic examinations and x-ray plates to aid the student in checking the accuracy of inspection and percussion.

In conjunction with supervision by competent instructors, it should prove to be an excellent supplement to clinic and bedside demonstrations.

P. P. S.

Gray's Anatomy of the Human Body. By HENRY GRAY, F.R.S. Fellow of the Royal College of Surgeons; Lecturer on Anatomy at St. George's Hospital Medical School, London. Twenty-second Edition, thoroughly revised and re-edited by Warren H. Lewis, B.S., M.D., Prof. of Physiological Anatomy, J. H. U. Balto., Md. Research Assoc., Carnegie Inst. of Washington. Illust. with 1232 Engravings. 1391 pp. 1930. (*Lea & Febiger, Phila., Pa.*)

The twenty-second is a marked improvement over the previous editions, chiefly because it is profusely illustrated with drawings both in color and plain. The subject-matter has been entirely revised and brought up to date. Much of the newer material on the central nervous system has been added.

This edition should prove all the more to be the choice of American Students in Anatomy.

P. P. S.

The Black Death and Men of Learning. By ANNA MONTGOMERY CAMPBELL. XII + 210 pp. (*New York, Columbia University Press, 1931.*)

In the period 1200–1350, there was a great upthrust of cultural activity and achievement in Europe, well nigh as remarkable as the Renaissance and Revival of Learning, with which it might have been continuous. It was the age of Dante, Petrarca, Giotto, Roger Bacon and Mundinus, of the Salernitan and later Latin surgeons, of Magna Charta and the laws of Frederick II for the improvement of medical education, of Oxford, Cambridge, Padua, St. Côme and the Sorbonne, of the introduction of spectacles, firearms and the mariner's compass, of the movement for shelter and treatment of the sick in hospital. Yet about the middle of the 14th century, this vast cultural wave collapses and trails off into a full century of comparative sterility, persisting even beyond the invention of printing (1438–50) and the fall of Constantinople (1453). Whence this solution of continuity, whereby 13th century achievement seems a kind of false dawn or aborted Renaissance? One explanation of the phenomenon is attempted in this book. The usual explanation is the Hundred Years War (1346–1453), which bestrides the debatable period so exactly that other factors seem negligible. But two years after the battle of Crécy (1346) came the Black Death (1348), which spread all over Europe in less than two years' time, destroying anywhere from one-fourth to nine-tenths of a population in different areas. One effect of the great war and the "Great Mortality," as the pest epidemic was called, was the improvement of the social and financial status of the working classes, whose services came to be all the more in demand through the general wiping out of the total population. Another effect of the Black Death, as our author shows, was the decline of learning, of university activities and even of common school instruction, through the depletion of teachers and pupils alike. The facts and figures adduced, including the high mortality among physicians and other intellectuals of the period, are quite conclusive. During 1348–1400 there were five major epidemic waves of the pest and the end was not yet, even with the Great Plague of Milan (1630). Transmission of the infection (by rat and flea) was swift and overwhelming, as in the epidemic of 1918–1919, of

which Vaughan observed that "when influenza strikes a nation, *all* are susceptible. The psychologic effect upon the population was mortuary, a tense feeling of impending doom, of the brevity, vanity and impermanence of human life and the necessity of preparation for the Heaven or Hell beyond the grave. This general state of mind was intensified to the highest point among the intellectuals. Comparing the pestilence of 1348 with the wars of the period, Sassoferrato, a jurist of Perugia, opines that "the hostility of God was stronger than the hostility of man." Three hundred years later (1653), the English dramatist Shirley summarizes as follows:

> "Devouring Famine, Plague and War,
> Each able to undo mankind
> Death's servile emissaries are."

The author does not develop her theme with the skill and precision manifest in such books as Prinzing's "Epidemics resulting from Wars" or Parkes Weber's "Death in Art." Her analysis of the content of the plague tracts of 1348–50, covering 87 pages, is valuable, but diverts the reader's attention from her basic thesis, and is not really essential to the *Durchführungssatz*. Her thesis, in fact, could have been developed to better purpose in a small fraction of the pages employed. Nevertheless, with patience and attention, the reader will gain a just notion of the capacity of a major epidemic to "undo," on occasion, even the intellectual activities of mankind.

F. H. G.

The Physiology of the Vestibular Apparatus. By MARIO CAMIS; translated and annotated by R. S. Creed. xiv + 310 pp. Cloth, $7.50. (*Oxford University Press, London, 1930.*)

The author of this book is the Director of the Institute of Physiology of the Royal University of Parma, and the translator is a Fellow of New College, Oxford. Both have been pupils of Sir Charles Sherrington, to whom this English edition is dedicated.

An enormous amount of work has been done in this field by numerous competent investigators. The bibliographical list alone occupies 39 pages of the book. It includes only papers with a physiological bearing, omitting much of the anatomical and clinical work; and even with this limitation does not claim to be complete. The author undertook a truly formidable task when he attempted to reduce to the confines of a moderate-sized book the essence and many of the details of the efforts which have been made to solve the numerous complex problems involved. Much of the account is coloured by the rather positive views of the author himself, but in the opinion of the reviewer this can only be regarded as a proper function of a critical summary. The subject matter is too complex to be even summarized here. Because of the intimate association of the vestibular apparatus with equi-

librium and motor coördination many people who are not particularly interested in the ear itself will welcome this book, and especially so since a summary of such scope has not previously been available. The format, press-work and illustrations are excellent.

S. R. G.

Recent Advances in Allergy. (Asthma, Hay-Fever, Eczema, Migraine, etc.) By GEORGE W. BRAY, M.D., Ch.M. (Sydney). 98 illust., includ. 4 colored plates. xii, 432 pp. (*P. Blakiston's Son., Phila., 1931.*)

Orienting oneself through the maze of recent studies in allergy is often difficult if not almost impossible. Dr. Bray's book practically removes this difficulty. The literature in question is carefully reviewed and classified, etiology is discussed in a critical forward-looking manner, and treatment is taken up in sufficient detail to be of service to the practitioner.

One's impression from reading the book is that the author has done his work conscientiously. The material is not merely tabulated as such, but presented in a manner to guide one away from the doubtful toward the more trustworthy. The book should prove of distinct value to the clinician and to the investigator.

E. M. B.

A Manual of Practical Vertebrate Morphology. By J. T. SAUNDERS and S. M. MANTON. viii + 220 pp. $5.00. (*Oxford, at The Clarendon Press, 1931.*)

The avowed purpose of this manual is to provide the student with a description of selected details of the anatomy and morphology of certain vertebrate types to be dissected in an elementary course in Zoölogy. The material considered consists of lamprey, skate, whiting, roach, salamander, frog, lizard, snake, pigeon and sheep. One short chapter is devoted to the skull in the Mammalia and another to the osseous features of the trunk in Vertebrates. The final chapter discusses the osteology of the appendages, including the limb-girdles.

The illustrations, while not numerous, are mostly well considered and adequately presented, and the authors have packed a great deal of useful information into a limited number of pages. One wishes, in fact, that the limits of the book did not impose such conciseness and relative or complete neglect of many features which to others may be of equal importance to those included. Thus no mention of the muscular system is made, nor of the nervous system of the Reptilia. In addition, the treatment accorded some of the cranial nerves, especially V and VII, of the lower vertebrates will be found confusing for the elementary, and even the advanced student. This arrangement is in accordance with function, which is deemed "much more satisfactory than the older one of 10 to 12 cranial nerves (the nomenclature of which is retained for obvious reasons of convenience)."

The format of the book is excellent and it will be an asset to anyone's library.

A. B. H.

The Great Physician. A Short Life of Sir William Osler. By EDITH GITTINGS REID. pp. 299. (*Oxford University Press, London and New York, 1931.*)

Mrs. Reid has rendered a great service to the average reader of biographies who wishes to know the general outlines of Sir William Osler's life, but who has not time to assimulate the complex material presented in Dr. Harvey Cushing's two large volumes. Mrs. Reid, moreover, has done her work well, for she has brought to her task not only a first hand knowledge of all that Sir William wrote, but also a personal knowledge of what he was. Her book contains some illustrations that are interesting and new. She divides Sir William's life into twelve chapters, beginning with the "Formative Years" and the "Laying of the Foundation." Next she describes Sir William's career at Montreal, at Philadelphia and during his first years at the Johns Hopkins. Then follows an interesting chapter on the Johns Hopkins Medical School itself. The last part of the book deals with Sir William's first years at Oxford, his work during the War and with Revere Osler's death. The period that followed the loss of his son were years in which Sir William seemed to be living largely in the past, like a man who has been crippled by the loss of some integral part of his own personality.

J. R. O.

The Clinical Aspects of Venous Pressure. By J. A. E. EYSTER, B.Sc., M.D. (*The MacMillan Company, New York, 1929.*)

In this volume the author has brought together the results of many years' studies dealing with venous pressure, the importance of which he has done so much to emphasize, and has given us an altogether satisfactory text. The principles involved are discussed at length with due consideration of the influence of the various factors concerned in conditions associated with failure of the cardiac reserve, as well as in various other conditions not necessarily associated with cardiac failure. In the chapter devoted to the discussion of cardiac decompensation, the author emphasizes the significance of the double set of peripheral vessels of the liver as of great importance in the wide range of capacity of this organ as a blood reservoir. He also calls attention to the function of the spleen in this connection and emphasizes the fact that pulmonary edema is indirectly influenced by the venous engorgement and high capillary pressure in obstruction of the venous return flow from the kidneys. The various clinical aspects of venous pressure are described at length and the method of procedure to be followed in making the determinations is described with sufficient detail. A large number of illustrative clinical cases are included and a very important bibliography is added. This volume is to be heartily recommended to those desiring a text containing within moderate compass the significant points dealing with the subject.

E. P. C.

An Introduction to the Literature of Vertebrate Zoölogy. By CASEY A. WOOD. 4to, pp. xix + 643. (*Oxford University Press, London, 1931.*)

This large volume constitutes mainly a briefly annotated catalogue of the books.

periodicals and papers, dealing wholly or in part with vertebrates, which have been brought together in the extensive libraries of McGill University. These collections include an admirable array of incunabula, an unusually large series of fundamental treatises and over five thousand original drawings by animal painters. They are particularly rich in ornithological literature, but other fields of vertebrate zoölogy are also represented by an enviable wealth of titles.

In the first section of the book (146 pages) Doctor Wood gives a historical account of the rapidly increasing literature on vertebrates from Aristotle to the authors of today. He emphasizes the rôle of natural history societies, museums, extension expeditions, etc. in augmenting and advancing this literature. The strict, historical enumeration of publications is occasionally interrupted by sketchy biographies of authors or by remarks concerning some theories.

The small, second section of this *Introduction* forms a convenient key-index for a rapid and full use of the large third section, which is the catalogue proper, containing about thirteen thousand titles.

This last section is unquestionably very useful for work in all the many divisions of the vast field of vertebrate zoölogy and especially in the history of this science. This catalogue will also be welcomed by librarians as well as by students of the history of medicine since a large part of the ancient and medieval literature has been contributed by physicians.

The author deserves great credit for having undertaken the enormous task of compiling this "Introduction." One can not dismiss the thought, however, that it might have been even more desirable to spend the same amount of work and expense on a complete catalogue of the literature of a more restricted field of zoölogy of, if necessary, a more limited period of history, rather than on this more ambitious but quite incomplete and somewhat unevenly selected compilation based upon the, naturally, limited collections of the McGill libraries alone. Were other libraries to issue corresponding catalogues this would entail a tremendous amount of wasteful repetition.

A. H. S.